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COMPARATIVE BIOCHEMISTRY

Volume I: Sources of Free Energy

COMPARATIVE BIOCHEMISTRY

A Comprehensive Treatise

Volume I: Sources of Free Energy, 1960

Volume II: Free Energy and Biological Function, 1960

Volume III: Constituents of Life—Part A

Volume IV: Constituents of Life—Part B

Volume V: Cells and Organisms—Part A

Volume VI: Cells and Organisms—Part B

COMPARATIVE BIOCHEMISTRY

A Comprehensive Treatise

Edited by

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Volume I

SOURCES OF FREE ENERGY

1960

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PREFACE

The aim of this Treatise on Comparative Biochemistry is to provide a sound, critical, and provocative summary of present knowledge in the field. Although comparative biochemistry is the most recent approach to the study of biology, its domain is vast because it seeks to compare the physicochemical properties of every form of life. In this sense it includes all of biochemistry—not only those aspects common to life in general, but also the unique physicochemical manifestations which are characteristic of each of the several million species of living organisms comprising the phylogenetic scale. Biological diversity is inherently as interesting to comparative biochemistry as biological unity. Furthermore, comparative biochemistry is concerned not only with contemporary life, but with life of all ages reaching back to origins, it views evolution in terms of molecular rather than morphological changes.

Several excellent pioneer monographs on comparative biochemistry have been published but none is more than an introduction to the field. This certainly is no criticism, because the time and scholarship required by a single author to survey critically the whole subject would surely dwarf the effort expended by Darwin himself on the much more meager information which provided the foundations for the "Origin of Species" one hundred years ago. Consequently, each phase of Comparative Biochemistry has been written by an author critically selected for his competence in the subject with which he deals.

The organization of the Treatise has been of particular importance and concern because only systematic division of the field into well-related groups of subjects can provide a framework for comprehensive treatment of the whole subject with minimum overlap between chapters. In our concern for the integrity of the Treatise's organization and its utility to the reader, we have preferred to delay publication of individual volumes rather than have chapters appear out of their organizational context. However, when delay of this kind has occurred, every effort has been made by the Publishers to give authors the opportunity to incorporate the most recent information in their chapters.

The authors contributing to this Treatise have had a particularly difficult task. In most instances their chapters have been pioneering efforts. The chemists among them have had to approach their topics, in part, from the unfamiliar ground of comparative biology, while the biologists have had to consider molecular dynamics more closely than they might otherwise have done. In the effort to achieve over-all consistency of purpose and outlook, many writers have patiently revised

their chapters. They all have had to brace themselves against the over-simplifying attractions of biochemical unity with the austerities of biochemical diversity.

In order to provide a systematic comparison of the biochemical phenomena of life throughout the phylogenetic scale, which has been our objective, the Treatise has been organized in the following manner: Volumes I and II are primarily concerned with the biological transformations of free energy. Volume I deals with the sources of biologically useful free energy, while Volume II describes how this free energy is utilized to support function. The structures of the principal classes of metabolites, their distribution, and the comparative enzymology of their biogenesis and metabolism are the subjects of Volumes III and IV. The comparative biochemistry of organized reaction systems and the biological functions dependent upon these systems are discussed in Volumes V and VI.

The chapter on Comparative Biochemistry of Photosynthesis, by Professor E. C. Wassink, which was scheduled to appear in Volume I, will be published in a later volume.

The Editors were saddened by the deaths of three distinguished contributors, Professor E. Newton Harvey of Princeton University, Professor C. Fromageot of the University of Paris, and Professor W. Bergmann of Yale University. They possessed unique commands of their fields of interest, and we are fortunate to be able to include their chapters on comparative aspects of bioluminescence, inorganic metabolism, and steroid distribution.

We wish to record our gratitude to the staff of our Publishers, Academic Press, for warm support and professional assistance throughout the preparation of the Treatise. They have made the task of editing far less onerous than it might have been. One of us (H. S. M.) wishes to thank Professor Sir Alexander Todd for the hospitality of his Department at the University of Cambridge during the editing of Volumes I and II, and Professor E. S. West of Portland, and Mr. T. N. Morris, M.A., of Cottenham, Cambs., for helpful editorial suggestions.

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May, 1960

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A Guide to the Nomenclature and Classification of Organisms

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Since the time of Linneaus, all organisms have been given Latin specific and generic names, the latter including groups of species more closely related to each other than to other groups of species. Even before evolution was accepted as a valid theory, biologists realized that genera could be arranged in a series of higher or more inclusive groups, denoting further interrelationships, on the basis of sets of characteristics held in common. Such classification systems tended to be artificial ones, but as more information concerning morphological, physiological, and biochemical characteristics of organisms was assembled, more natural systems were devised. Once evolution was accepted, these natural classification systems reflected the phylogenetic picture pieced together from paleontology, and from the study of the comparative aspects of modern forms.

The basic conflict between partly artificial classification systems, often more complete and more useful for purposes of strict identification, and the natural phylogenetic systems, sometimes less practical for taxonomic purposes, has been discussed frequently (*1–4*). Furthermore, a phylogenetic scheme suitable for modern forms might be quite unsatisfactory if fossil forms are included. As the present treatise is concerned with the comparative biochemistry of extant forms, any purely fossil groups are not included in the classification scheme presented, although such fossil forms often are the basis of any phylogenetic scheme.

While numerous subdivisions and even higher taxonomic categories are frequently used, the following taxa, arranged in order of decreasing magnitude, are the generally accepted groupings used to classify related organisms:

Phylum (or Division in the Plant Kingdom)
  Class
    Order
      Family
        Genus
          Species

The diagram (Chart I) summarizes one viewpoint of the phylogenetic relationships between the phyla or divisions of extant organisms. Such a chart, derived mainly from Hyman (5) and Smith (6), is presented merely as a guide to present day phylogenetic theories, and should serve only as a useful working hypothesis. Although no two biologists would ever agree on all details of such a phylogenetic "shrub," this diagram represents a generalized view held by many biologists. While comparative biochemistry has already contributed to our knowledge of phylogenetic relationships, it is hoped that a better understanding of comparative biochemistry can either strengthen or weaken the bases on which such a phylogenetic scheme have been drawn.

The vertical axis is not a time axis, but represents either a crude measure of the relative morphological and physiological complexity, or the relative degree of evolutionary advancement of the phyla or divisions named. The time axis would extend downward in a perpendicular direction below the surface of this paper, and the inclusion of such an axis would necessitate a three-dimensional diagram. Many of the phyla or divisions placed here would extend as independently evolving lines well down in the scale of geological time to the Cambrian period. The Spermatophyta and the Chordata, on the other hand, arose more recently. Estimates of the time of origin of these major groups are given in Simpson (3) and Smith (6). The lines interconnecting these major forms reflect the evidence based on paleontology, and the comparative morphology, physiology, and biochemistry of modern forms. Groups that have been separated from each other for a long time, and which have been evolving independently during this entire period, appear in the widely diverging lines such as those separating the higher plants from the higher animals. A more recent separation is that of the Aves and Mammalia from a reptilian ancestor. In some cases there are known fossil precursors at the positions just below a main fork, such as the Psilophytales in the line leading to the radiation of modern vascular plants (Tracheophyta) and ending in the Spermatophyta. In other cases, the common precursor below a fork is a hypothetical group postulated on the basis of comparative studies of modern forms. This is the case in the primitive acoelomate Bilateria which evolved into the two major lines, the Protostomia and Deuterostomia.

The "shrub" effect at the base of the diagram indicates a polyphyletic origin from a hypothetical complex of primitive organisms. The arrangement of the phyla at the base emphasizes the artificiality of the groups often referred to as Protozoans, Algae, and Fungi, and the difficulty in assigning many of the phyla of microorganisms to either the

plant or animal kingdom, except on an arbitrary and artificial basis. Certain organisms, furthermore, are classified as both plants and animals; i.e., *Euglena*, *Volvox*, etc. For convenience only, the green flagellates of the Protozoa and their colorless counterparts are best placed in the Euglenophyta or Chlorophyta, leaving the more highly specialized colorless flagellates in the Protozoa. Most of the interrelationships between these groups of microorganisms are quite indistinct, indicated by the dashed lines, but it is felt that any grouping of the algae and fungi in one group, the Thallophyta, is highly artificial, and that they represent many separate stocks that have been evolving independently for a long time.

Such a phylogenetic chart can be used as a guide to examples of parallel or convergent evolution occurring in these major evolutionary lines. For example, any morphological or biochemical characteristic found only in the Mammalia and Insecta is probably the result of an independent origin in the two groups, while a characteristic found in all the main Chordata and none of the phyla along the main line leading to the Arthropoda would presumably have originated once sometime after the separation of the two groups indicated by the fork. The step-by-step loss of morphological and biochemical characteristics along any one line is possible, and a simple structure or function may be the result of reduction rather than a result of a primitive condition.

The major phyla or divisions in the plant and animal kingdoms are again listed in Charts II and III in tabular form along with the approximate number of species within a phyla, and some of the synonymous names or other taxonomic categories that might help in making cross-relationships between different classification schemes. Further aid in the placement of organisms within these listed phyla can be obtained by reference to the sources cited below.

Although it would be preferable to use only one classification system down to the species level for a treatise like the present one, this is not yet practical. While there are some controversies concerning even the major groups shown here, the differences in opinion become even greater in the lower groups. For taxonomic classification systems of taxa below the phyla level, the authors will cite their taxonomic source when necessary. Because of possible misinterpretation if only common names are used, the genus, and if known, the species names will be given.

Reasons for the existence of so much controversy concerning not only the group into which an organism should be placed, but the taxonomic rank to which different groups should be elevated have been frequently discussed (*2–4*). Although some of these difficulties are

caused by a lack of information concerning certain groups, others are inherent in the fact and mechanisms of evolution itself. While some minor differences still remain, geneticists and experimentalists have defined the taxon (or group) called a species in sexually reproducing organisms in terms of the amount of gene exchange still occurring, a factor which can be tested experimentally. Therefore, a well delimited population of individuals which possesses a set of distinguishing morphological, physiological, or biochemical characteristics can be assigned the taxonomic rank of a species if there is still gene exchange occurring within the group, but not with closely related groups (*2–4*). Unfortunately, similar definitions for families, orders, etc., are not available, and although a higher taxonomic group might be equally well delimited and represent a natural grouping, the rank assigned to that group is relatively arbitrary (*2–4*). Any evaluation of these taxa, therefore, is determined mainly by its convenience and usefulness, and might vary with different workers. Families, classes, orders, etc., in different evolutionary lines, therefore, will not be comparable to each other in terms of evolutionary advancement, size, etc. In other words, organisms have evolved into different species; after they have become well defined, biologists arrange them into higher and higher degrees of order and magnitude to illustrate the interrelationships.

The longer that groups have been separated in evolutionary time, the easier it is to distinguish between or to delimit the taxonomic groups involved. However, even here, there will still be "splitters" and "lumpers" among taxonomists, and indecision as to the rank of even a well delimited group. On the species level, furthermore, the genetic definition is often impractical, and taxonomists will separate certain groups into a number of species although some gene exchange is still occurring. The synonymous terms indicated in Charts II and III illustrate some of these problems on the phylum level. Smith for instance, prefers to elevate the ferns (Pterophyta) to the rank of a separate division, while others have grouped them in the subdivision Pteropsida along with the Gymnospermae and Angiospermae. Hyman, likewise, discusses the splitting and lumping of many of the small groups of the pseudocoelomate forms within the Bilateria.

The following references are listed as sources of further information discussed here.

R. S. Breed, E. G. D. Murray, and A. P. Hitchins, eds., "Bergey's Manual of Determinative Bacteriology," 6th ed. Williams & Wilkins, Baltimore, Maryland, 1948.

F. A. Brown, Jr., ed., "Selected Invertebrate Types." Wiley, New York, 1950.

P. Grassé, ed., "Traité de Zoologie," 17 volumes. Masson, Paris, 1948.

A. S. Romer, "The Vertebrate Body," 2nd ed. Saunders, Philadelphia, Pennsylvania, 1955.

K. V. Thimann, "The Life of Bacteria." Macmillan, New York, 1955.

Summary

1. The Charts are hypothetical schemes showing the relationships between major taxa of extant organisms, and represent one possible picture based largely on evidence from paleontology and the comparative morphology of living forms. The composite chart (I) has been derived mainly from Hyman (5) and Smith (*6*), and represents a generalized opinion held by many biologists. No two biologists, however, would ever agree on all details of such a chart.

2. The artificial classification systems such as those summarized in the "Handbook of Biological Data" (pp. 527–533) (*7*) are more inclusive (and therefore, more useful for identification purposes), but they fail to show our present day concepts concerning phylogeny.

3. The phylogenetic chart, then, is presented as a guide to present day concepts of phylogeny, and should serve only as a useful *working hypothesis.*

4. Aid in the placement of organisms in either the artificial (*7*) or the above phylogenetic scheme, and the explanation of synonymous terms can be found in the references listed at the end.

5. Note that some forms within the microorganism complex are classified as both plants and animals: i.e., *Euglena, Volvox,* etc. For convenience sake, the Flagellata (Mastigophora) of the protozoans can best be placed in the Chlorophyta or Euglenophyta [see Smith (*6*)].

6. Note that little emphasis should be placed on the relative level of any taxonomic group above the species or perhaps the genus level. Only the species can be defined in a nonarbitrary fashion (*3, 4*). Other taxa are still arbitrarily designated. In other words, families, classes, orders etc. in different evolutionary lines are *not* comparable to each other in terms of evolutionary advancement, size, etc. (*3*). The largest group is generally called a phylum in the animal kingdom and a division in the plant kingdom.

References

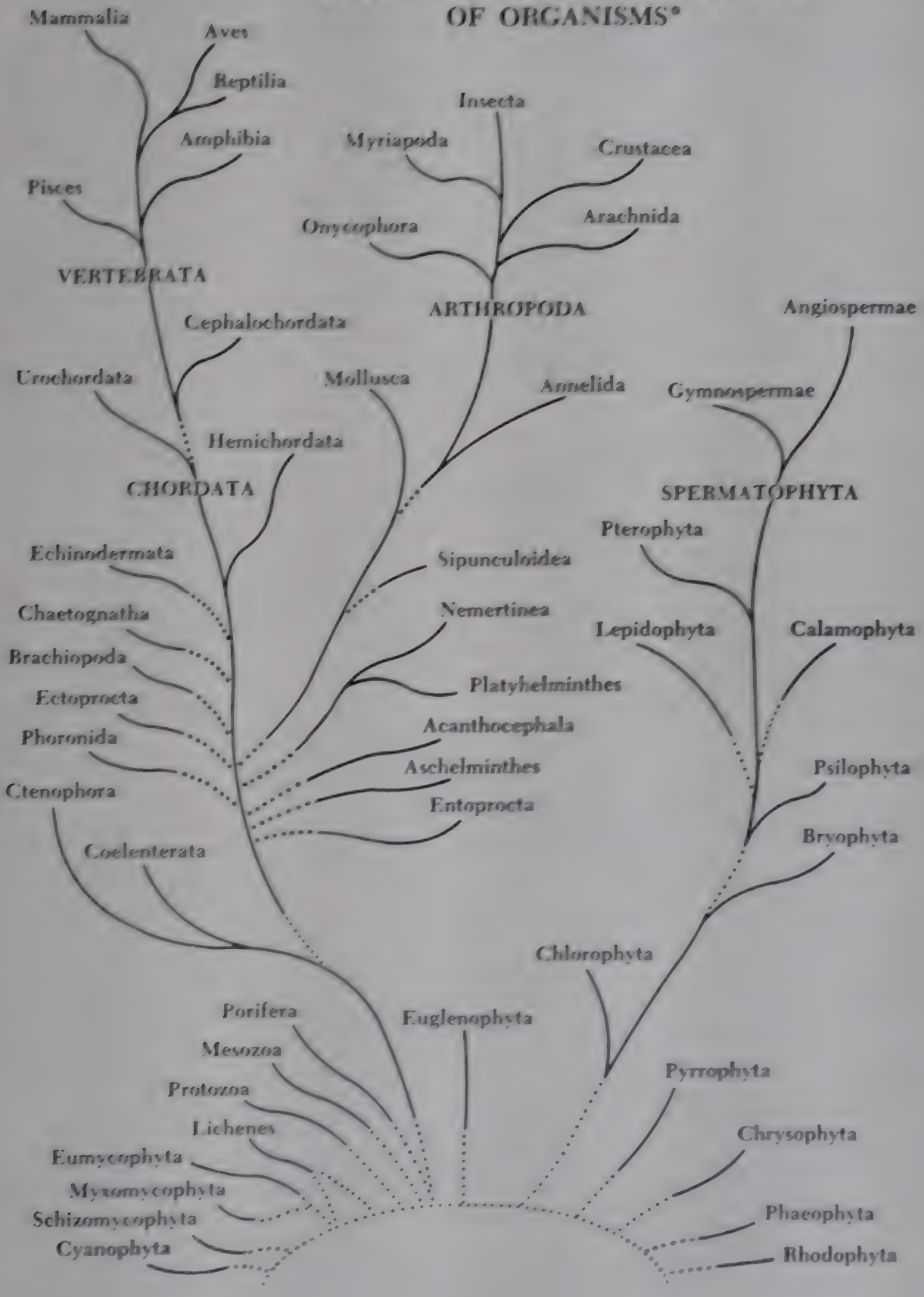
1. G. H. M. Lawrence, "Taxonomy of Vascular Plants." Macmillan, New York, 1951.

2. E. O. Dodson, "A Textbook of Evolution." Saunders, Philadelphia, Pennsylvania, 1952.
3. G. G. Simpson, "The Major Features of Evolution." Columbia Univ. Press, New York, 1953.
4. G. L. Stebbins, Jr., "Variation and Evolution in Plants." Columbia Univ. Press, New York, 1950.
5. L. Hyman, "The Invertebrates," Vols. I–IV. McGraw-Hill, New York, 1940–1956.
6. G. M. Smith, "Cryptogamic Botany," Vols. I and II. McGraw-Hill, New York, 1955.
7. W. S. Spector, ed., "Handbook of Biological Data." Saunders, Philadelphia, Pennsylvania, 1956.

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* Most of the names refer to phyla, except in a few cases where some of the smaller taxonomic groups are shown. Capitalized names written across lines are groups including all forms above the name.

CHART I
HYPOTHETICAL PHYLOGENETIC RELATIONSHIPS BETWEEN EXTANT MAJOR GROUPS OF ORGANISMS*



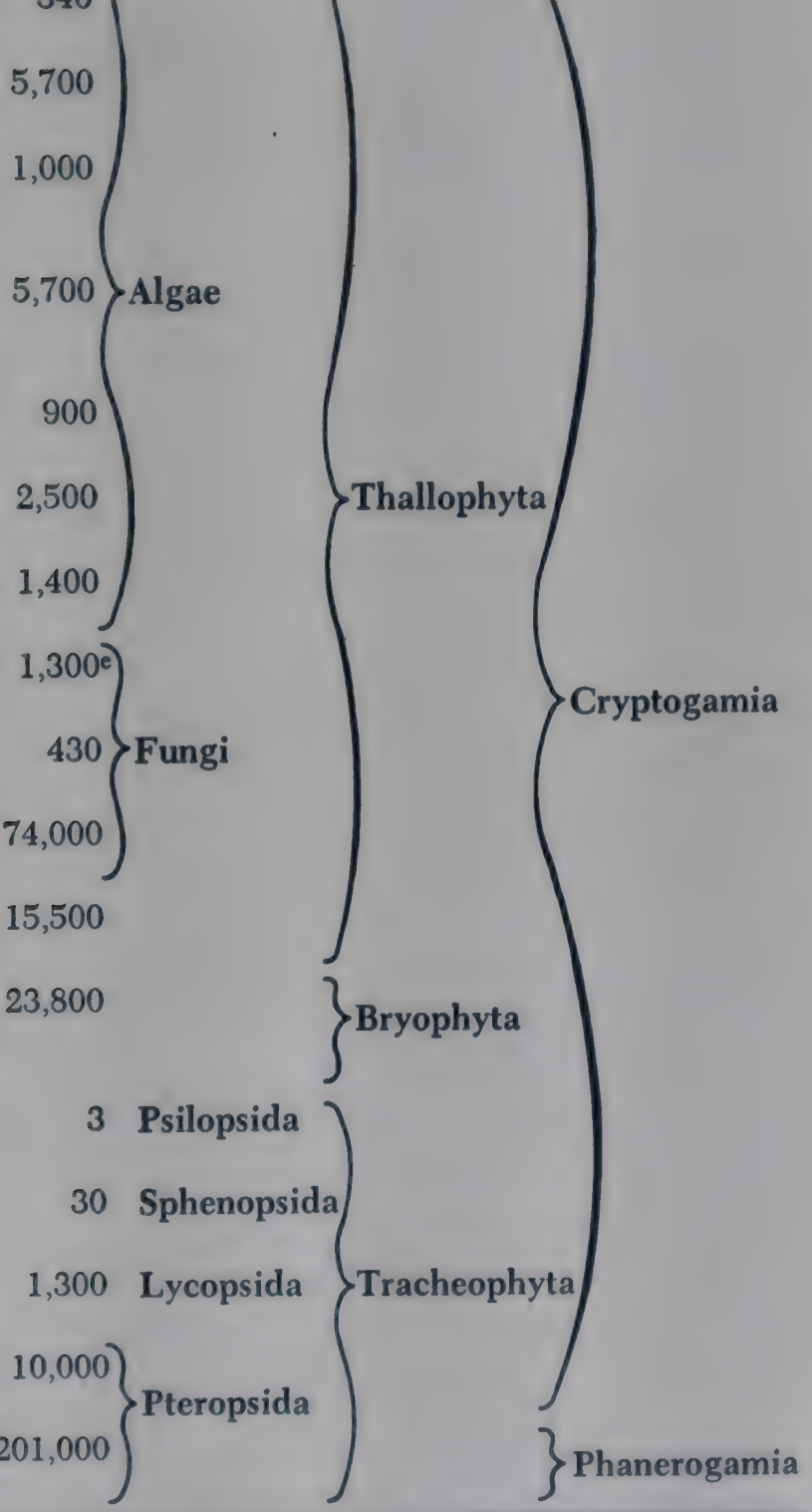
Mesozoa	–		
Porifera (sponges)	5,000		
Coelenterata (coelenterates)	10,000		
Ctenophora (comb jellies)	100		
Platyhelminthes (flat worms)	6,000	Acoelomates	
Nemertinea (nemertine worms)	500	Acoelomates	
Aschelminthes[a]	7,000	Pseudocoelomates	
Acanthocephala[a]	7,000	Pseudocoelomates	
Entoprocta[b]	3,000	Pseudocoelomates	
Ectoprocta[b] (moss animals)	3,000	Pseudocoelomates	
Phoronida	15	Eucoelomates	Schizocoela
Brachiopoda (lamp shells)	120	Eucoelomates	Schizocoela
Mollusca (mollusks)	70,000	Eucoelomates	Schizocoela
Sipunculoidea	–	Eucoelomates	Schizocoela
Annelida[c] (segmented worms)	6,500	Eucoelomates	Schizocoela
Arthropoda (arthropods)	750,000	Eucoelomates	Schizocoela
Chaetognatha (arrow worms)	30	Eucoelomates	Enterocoela
Echinodermata (echinoderms)	5,000	Eucoelomates	Enterocoela
Hemichordata	60,000	Eucoelomates	Enterocoela
Chordata (including vertebrates)	60,000	Eucoelomates	Enterocoela

[a] Includes Rotifera, Gastrotricha, Kinorhyncha, Nematodoidea. Formerly called Nemathelminthes.

[b] Formerly in Bryozoa.

[c] Includes Echiuroidea.

[d] Taken from "Handbook of Biological Data" (*4*), p. 533.


5,700
1,000
5,700 Algae
900
2,500
1,400
1,300e
430 Fungi
74,000
15,500
23,800
Bryophyta
3 Psilopsida
30 Sphenopsida
1,300 Lycopsida
Tracheophyta
10,000
Pteropsida
201,000
Thallophyta
Cryptogamia
Phanerogamia

chizophyta.

CHAPTER 1

An Introduction to Comparative Biochemistry

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I. The Comparative Point of View in Biology

The phenomena of life are so numerous and so diverse that no human mind, whatever its genius, has been able to arrange them entirely within the framework of a single classification. Nevertheless attempts to do so have been made since ancient times. The objective knowledge of life acquired by the world of antiquity was systematized by Aristotle. During the two thousand years which followed, dogmatism and verbalism replaced inquisitiveness in a society into which no fresh sources of objective knowledge were admitted; but from the beginning of the sixteenth century, new experience forced new kinds of comparisons. Chief among these were the objects (and ideas) brought back to Europe by explorers, and the fantastic new world which was revealed by the discovery of the microscope. The character of these discoveries and observations was so striking that the techniques of collecting, drawing, and cataloguing became very important, and introduced an element of classification which had been lacking until that time. It became necessary to compare organisms with others of their kind and to revise notions of similarities and differences in the light of previously unsuspected knowledge. This flowering of Renaissance biology was not the product of any special human genius but was forced by observation. Thus, when anatomy became an exact science, i.e., after Vesalius, several physicians studied other mammalian species and compared them with man. The monograph of Luini on the horse, at the end of the six-

teenth century, is an example of this kind and was only one among many such studies. Severino, an anti-Aristotelian biologist, published his "Zootomia democritaea" in Nuremberg in 1645, and this book may well be considered the first systematic exposition of analogies in the structure of animals which was to become the basis of the concept of unity in design. The contributions embodied in numerous monographs on vertebrates found their way into the "Anatome animalium" (1691) of Blasius, the first systematic treatise of the science which had been called *comparative anatomy* by Nehemiah Grew in 1675. Grew himself had studied the relationship of various parts of plants throughout different groups.

The great comparative effort at the end of the seventeenth century brought with it a wealth of anatomical knowledge. As often happens, this new information was not only invaluable in itself but also became the basis of a new line of speculative thought. Charles Bonnet (1720–1793) developed a new form of the Aristotelian idea of the "ladder of nature" proceeding via molds, plants, insects, worms, fishes, birds, mammals, and man, all variants of the prototype which was man. From this concept there originated the *Naturphilosophie* which for a time turned comparative anatomy into a barren field. The *Naturphilosophen* had a unitarian view the purpose of which was to define the basic principle of nature. They were fond of "types," "schemata," "ideal forms," etc. One of these was the idea of the uniformity of animal design, a view which Aristotle had had in mind and which was much later reformulated by Goethe in his vertebral theory. Oken set this theory out more categorically. For Oken, man is the crown of nature, since he united in himself the five senses which enable him to learn about nature. The whole animal world was, then, comprised of five divisions of animals, each particularly endowed with one out of the five senses united in man.

A new line of comparative research undermined the extravagances of *Naturphilosophie*. When biologists began to study comparative embryology they obtained a much deeper knowledge of the relationships between corresponding parts of organisms, and it became possible to develop the concept of homology. For instance, Rathke (1743–1860) discovered structures in embryos of birds and mammals which were homologous with gill slits in fish embryos.

Another comparative study was that of the taxonomists who were interested in classifying and naming living organisms. Such a classification must enable a naturalist to identify a plant or animal, dried or preserved in alcohol. Hence it is unfair to reproach taxonomists for giving so much attention to external details of structure. John Ray

(1627–1705) and Karl Linnaeus (1707–1778) collected a number of comparative data from which they built their classificatory systems and nomenclatures—a work which has been extended by many successors and which is not yet complete as new animals and plants are constantly being discovered.

The work of taxonomists has been a rich source of biological concepts, and it is worthwhile to go back to the origins of classificatory activities based upon the comparison of living forms. From the comparisons made by Aristotle, two conclusions appeared: in nature, there are "genera" displaying a real consistency; and in order to identify genera exactly it is necessary to pay attention to all characters which, taken one by one, might either separate or unite them. Taxonomy is, therefore, a mirror of the state of empirical knowledge of classified organisms.

The method introduced by Linnaeus into zoology was marked by a categorical affirmation of the natural reality of species and genus. For him, species and genera were inherent in nature, to be discovered by observation. His method and the consequent results brought Linnaeus to a speculative concept of wholeness according to which the living world, or as we would say today, the biosphere, is a well ordered whole in which each class realized a part of the possibilities allowed by God and accomplished this role in equilibrium with the roles of the other classes.

If, at the end of the eighteenth century, the "methods" of taxonomy were considered necessary, many biologists were unsatisfied with the acceptance of a taxonomic system as representing the fundamental relationships between organisms. A new, fruitful and decisive advance was then introduced by George Cuvier (1769–1832) based upon a mass of new comparative data. For Cuvier, the work of nomenclature ("die gar kein Kopf erfordert" as he writes to his German friend Pfaff in 1788) provides only the lexicon of natural history but does not teach the language. His first tendency was to study the forces and functions for which the great diversity of forms and functions serve as instruments. But at that stage his concept of physiology was in fact, the concept of *anatomia animata*. Cuvier, in agreement with the current views of his time concerning the "economy" of the interrelationships among organisms, had in mind a thorough study of functional interrelationships, of mutual (physical and physiological) interactions of organisms, and a search for an explanation built upon the internal mechanisms in each organism. Until then, anatomy had been the domain of medical men. It was quite a new development for a zoologist to look for comparative aspects of animals with scalpel and forceps. The de-

velopment which started with Cuvier was that of the comparative study of internal organization. Dissection became an important method of comparative enquiry. Dissection and classification went hand-in-hand, it was from the internal organization that true distinctions among classes had to be derived.

In spite of the new character of his comparative enquiry, Cuvier and his followers nevertheless remained tenants of the past in the degree to which they classified and determined the places of organisms in a reticulum of resemblances: they were not observers of living organisms, performed no experiments to determine the function of an observed apparatus, made no reference to development, and made no use of magnifying devices. For Cuvier, the "character" which determined an order was a fact of organization which, by the condition it imposed upon the life of the animal which possessed it, completely determined the complexity of its organs and the nature of its physiological activity.

The general classification proposed by Cuvier in 1795 was a considerable advance over the division of animals based upon identification of physiologically dominating organs. Diversity was expressed by Cuvier in nine "classes": mammals, birds, reptiles, fishes, mollusks, worms, crustaceans, insects, and zoophytes. Mammals were the most perfectly organized, with their hearts and complete vascular system. For Cuvier, man was the basis of reference with respect to which all other "classes" were to be analyzed and understood. The studies on comparative physiological anatomy of which Cuvier was the initiator made untenable the theory that organisms could be arranged in a single continuous line. Cuvier separated animals into four phyla, characterized by four different general plans into which animal structures seem to have been molded. Each subsequent division of phyla was based only upon small modifications consisting of the development or the addition of some parts which left unmodified the essence of the plan. The four phyla (Vertebrates, Mollusks, Articulates, and Zoophytes) were not characterized by physiological properties, as the classes were, but by morphological properties, as the names "Articulates" and "Zoophytes" attest. For Cuvier, there were no transitions between the four phyla, no universal identity of plan, nor any series. If there was a unity, it was not a unity of organs, but a unity of functions.

On the other hand, Lamarck (1744–1829) understood the "natural order" as gradual and continuous progression. He was firmly opposed to the concept of a network of resemblances at the knots of which each species appears connected with innumerable relations. According to

Lamarck, the studies performed by Cuvier and his disciples demonstrated a scale of animals in growing complexity of their organization. For him, the resemblances were the effects of a genetic derivation and not, as they were for Cuvier, evidence of a community of nature.

The "unity of plan" dear to Goethe and to Geoffrey St.-Hilaire was a purely philosophical concept. It was not the only endeavor to define organic unity. To this natural question in the human mind, a scientific answer was given by the cell theory as formulated by Theodor Schwann in 1839. As this theory remained the basis of organic unity until the present time, it is worthwhile to examine how comparative studies brought it about. The search for a common principle of organization had been the aim of micrographers for some time. In the eighteenth century, the unit was the "fiber." Later on came the "globulists" such as Fontana, Wenzel, Dutrochet, Raspail, and Purkinje. The notion of "globule" included quite a variety of structures: grains, nuclei, as well as optical illusions, but occasionally the "globulists" came across a real cell. The botanists were first to realize that plants were exclusively made of cells but at the time that Schwann wrote his epoch-making book, the general opinion was that the growth of animals essentially differs from the growth of plants. According to ideas then current, the presence of blood vessels was characteristic of animals and determined their mode of growth. This concept gave way to the theory proposed by Schwann, according to which all organisms are formed by anatomical elements derived from cells, and are cells, differentiated cells, or the products of cell activity. The original and valuable contribution of Schwann was to show the formation of all tissues by cell differentiation; his theory in which he adheres to Schleiden's principle that cells are produced in an unorganized medium has long been proved to be wrong. The theory that cells are produced by the division of pre-existing cells resulted from three lines of research: the observation by Trembley, Ch. Morren, Ehrenberg, etc. of the multiplication of Protista; the observation by Dumortier, Mohl, Meyen, and others of cell multiplication in Algae; and the observation by Prevost and Dumas, by von Siebold, by Bary, and by others, of the development of the egg.

But it was Robert Remak, a student of Johannes Muller, who generalized and clearly described the notion of cell multiplication by division of pre-existing cells, a concept which was popularized and applied to pathological growth by Rud. Virchow. The cell theory is not a philosophical theory. If it is true that Schwann, who tried to oppose the point of view of the physicist to that of the *Naturphilosophen*, found the very principle of unity which had been one of the objectives of *Naturphilosophie*, his main scientific discovery, brought about by his

comparative studies of cell differentiation, was a great scientific achievement and one of the greatest in the whole history of biological sciences.

The scientific definition of organic unity in the cell theory has been more and more determined by comparative studies and at present its form has taken on the content which J. R. Baker has stated as follows, in seven propositions:

"1. Most organisms contain or consist of a large number of microscopical bodies called "cells," which, in the less differentiated tissues, tend to be polyhedral or nearly spherical.
2. Cells have certain definable characters. These characters show that cells (*a*) are all of essentially the same nature and (*b*) are units of structure.
3. Cells always arise, directly or indirectly, from pre-existing cells, usually by binary fission.
4. Cells sometimes become transformed into bodies no longer possessing all the characters of cells. Cells (together with these transformed cells, if present) are the living part of organisms: that is, the parts to which the synthesis of new material is due. Cellular organisms consist of nothing except cells, transformed cells, and material extruded by cells and by transformed cells (except that in some cases water, with its dissolved substances, is taken directly from the environment into the coelum or other intercellular spaces).
5. Cells are to some extent individuals, and there are therefore two grades of individuality in most organisms: that of the cells, and that of the organism as a whole.
6. Each cell of a many-celled organism corresponds in certain respects to the whole body of a single protist.
7. Many-celled plants and animals probably originated by the adherence of protist individuals after division." (*1*)

The cell theory was the origin of many studies on histological comparative data and on the nature of the cell differentiations which compose an organism. But in spite of this rapid advance of knowledge at the level of the microscopical anatomy of organisms, the philosophical concept of the *order of nature*, either when considered as a gradual scale of organisms in a definite progression or when considered as an equilibrium of their mutual interactions, remained in the minds of naturalists who believed that the state under which the collection of organisms has its *raison d'être* is the very system of relations which constitute this collection. Darwin, through a new form of comparative enquiry and by considering organisms not in a herbal or museum but in the environment in which they live, put an end to the *order of nature*

and showed that the phylogenetic scale is the result of the temporal processes which lead to its existence. To the scientific idea of unity contained in the cell theory, the theory of evolution added the notion of a fundamental genetic unity of organisms. These two ideas favored the acceptance of the concept of General Physiology as a study of physiological phenomena common to organisms as proposed by Claude Bernard and also emphasized in Verworn's "Allgemeine Physiologie" (1894) and in Pütters "Vergleichende Physiologie" (1911). The latter writes that the scope of comparative physiology is "die Einheitlichkeit des vitalen Geschehens zu entfassen, das in so vielen heterogenen Formen uns in der Natur entgegentritt," a method in accordance with Claude Bernard's theory that "la Physiologie générale ignore les distinctions entre espèces, genres, familles, etc. . . . " In fact, General Physiology has become, as H. Davson recently stated, "the study of those aspects of living material that show some immediate prospect of being described in the known laws of physics and chemistry," a definition which comes near to that formulated by Claude Bernard: "La physiologie générale se trouve ainsi ramenée à être la science expérimentale qui étudie les propriétés de la matière organisée et explique les procédés et les mécanismes des phénomènes vitaux, comme la physique et la chimie expliquent les procédés et les mécanismes des phénomènes minéraux." Did not Cl. Bernard say, in a sentence which has been reported by Dastre, that "La physiologie générale, c'est la physique et la chimie de la matière vivante." In the light of our present knowledge it is clear that Bernard's General Physiology is nothing but cell physiology and the elaboration of the concept stated by Schwann of the autonomous physiology of the cell.

The same can be said of the discussion on the "unity of the biochemical plan" of organisms [Baldwin (*2*); Florkin (*3*)]. Since we know that the enzymatic systems of glycolysis and of the pentose cycle are generally found in the cytoplasm of all kinds of cells and that the enzymes of the Krebs cycle and of the cycle of fatty acids are generally found in the mitochondria of the cells of organisms of all kinds, it becomes clear that the concept of "unity of the biochemical plan" of organisms as well as the concept of "general physiology" have to be included in a modern formulation of the cell theory, as general cell biochemistry and general cell physiology.

Philosophical trends inborn in human nature recur over and over again in the human mind. There are no doubt many biochemists practising *Naturphilosophie* without knowing it, who believe that the scope of true biological science is to uncover all the mysteries of those chemical phenomena which are common to all cells, and thus reach a final

definition of life, holding as purely incidental the existence of pl algae, mollusks, fishes, mammals, or bacteria. Life is not an abst conceptual system but manifests itself in the same organism thr such deeply different biochemical systems as a liver cell and an dermal cell and through such biochemically different organisms cockroach and an oaktree. Biochemistry also hopes to bring its tribution to the understanding of this infinite diversity: "infinite" b the right adjective as, in biochemical as well as in other respects, living organism is a new reality.

If it is true that the cell theory and the theory of evolution r forced the unitarian trend of physiology, it also, in limited quar called attention to the consideration of diversity. This kind of empl had already been present in the writings of such outstanding natura as Geoffrey St.-Hilaire and Milne-Edwards. The study of physiolog diversity was greatly enhanced by the foundation by Anton Dohrn the Naples Zoological Station, of a physiological laboratory. As back as 1875, Dohrn, in an open letter to Von Siebold [*Z. wiss. Z.* **25,** 457 (1875)] underlines the fact that a theory of descendance, b only on a collection of data provided by comparative anatomy and comparative embryology, brings light only on one face of the prob and is only one of the factors to be taken into consideration. "I anderen bildet das Studium der Lebenweise und vergleichen Physiologie," as Dohrn writes. This appeal was heard by Preyer w in his "Elemente der allgemeinen Physiologie," published in 1883, t to introduce physiological considerations into the study of evolutio problems, a tendency which was immediately eclipsed by the succes Verworn's "Allgemeine Physiologie," written as we have stated, acc ing to a unitarian tendency.

Anton Dohrn felt it impossible to adhere to this. In 1867, in a le to Haeckel reproduced in his biography by Theodor Heuss (the for president of the German Republic), Dohrn clearly expressed his vi point on the necessary studies of comparative physiology to the be of the progress of phylogenetic problems: "Wie konnte aus diesen beschaffenen Organen der Vorfahren dies so ganz anders beschaffen Nachkommen hervorgehen? Die Kette der allmählichen Umwandlun muss ich erschliessen an der Hand physiologischer Hypothesen—u wie sollen diese Hypothesen einigen Wert besitzen, solange die wirk vorhandenen Funktionen all der verschiedener Meerestiere uns so bekannt bleiben, wie sie jetzt sind?" Since the beginning of the pres century, comparative physiology has collected a great amount of formation making clear many aspects of the mechanism of this or t organism and of the relation of the organism to its environm Physiologists are used to studying an organism in relation to one par

eter in the environment. As Preyer pointed out in his "Elemente," a physiological function should be considered in relation to a functioning apparatus and to a material substrate, for instance, a vascular system and liquids, a digestive system and aliments, a sensory system and a perceptible phenomenon. The relationship was that of the blowing wind and the windmill grinding the grain. In his comparative work directed towards knowledge of the diversity of organisms, Preyer insists on the idea that we should not compare embryonically concordant structures (the homologous), but functionally concordant structures, the *isodynamous* parts functionally equivalent in spite of differences of morphology and evolution. Preyer sought for a history of functions, and his series is to be understood as a classification of physiological innovations, according to their growing complexity but without reference to phylogeny. Thus, for example, he insisted on the importance of discovering transitions between the circulation of cytoplasmic granules and currents of blood.

Comparative physiological studies have also provided valuable information on phylogeny. For instance, the morphologists studied the transition of locomotion in Vertebrates, from aquatic to terrestrial life. A study of the morphological evolution of pentadactylous appendages with respect to transformations at the level of the skeletal apparatus and of the muscular apparatus resulted. Comparative physiological studies have, on the other hand, explained the complex mechanisms controlling not only the movements of the limbs, but also the relation of their position with the position of the organism itself. As Redfield says: "The fact that the fish's fin is primarily an organ of equilibrium rather than of locomotion is of the utmost importance, for it shows that long before the problem of standing or running arose in the terrestrial vertebrate, the neurological mechanism for postural co-ordination was laid down in the paired appendage." As it is only natural, morphologists and taxonomists in their comparative studies, have kept as their fundamental concept the notion of homology, i.e., the community of origin, while comparative physiologists, primarily concerned with the organism living in its natural ecological niche, have been mainly interested in the accomplishments and inventions of organisms. It is also quite natural that physiologists have gathered such valuable information on the many ways through which the different organisms solve the problem of their relation with a parameter of the medium.

II. The Comparative Point of View in Biochemistry

Thus, the great successes of experimental biology have led to recognition of structural and functional similarities and diversities among living organisms. A major contribution of the physical sciences

on the other hand, applied to life, has been to extend this understanding *to the molecular level.* From this extension has come two basic concepts which comprise the physicochemical foundations of comparative biochemistry.

The first of these concepts, formulated by Theodor Schwann, is that life is consistent with the fundamental properties of matter and energy, and is a consequence of them. From this point of view, the modern history of which commenced with Descartes and Hobbes about 350 years ago, it is matter and energy interacting at subatomic, atomic, and molecular levels which give rise to the dynamics from which the more highly organized aspects of biological unity and diversity are derived. These interactions also give rise to the dynamics of the universe. "The properties of matter and the course of cosmic evolution are now seen to be intimately related to the structure of the living being and to its activities" (*4*). Hence, the working hypothesis which, although rarely made explicit, underlies the activity of contemporary biochemistry, is that "the framework of a single classification," the fundamental properties of matter and energy, suffice to describe all the phenomena of life. The testing of this hypothesis is the most significant activity of comparative biochemistry, comparative in the sense that it studies the physicochemical consistencies between life and the universe in which it occurs.

The second comparative concept arising from the physicochemical study of life has been clearly stated by Baldwin: "We arrive from several different lines of approach at essentially the same conclusion: there exists a common, fundamental chemical ground plan of composition and metabolism to which all animals and very probably other living organisms also, conform, and that superposed on these foundations, there are numerous secondary, specific, and adaptational variations, some of addition and others of omission" (*2*). The expression "numerous variations" is perhaps an understatement because there exist at least two million species of plants and animals, among which the ultimate differences are almost certainly chemical (that is, in nucleic acid structure, although nucleic acids are common to all forms of life), added to which there are the secondary species, specific and adaptional variations, and biochemical variations (such as in human blood groups) which occur among individuals of a species. Nevertheless, as this Treatise will show, a common chemical ground plan (biochemical unity) is apparent in the midst of this diversity. That this is the case was foreshadowed in the development of nineteenth and twentieth century chemistry by the isolation of metabolites and the determination of their structures, by the discovery of enzymes, their proteinaceous struc-

tures and their relationship to metabolism and, most recently, by the resynthesis of metabolic systems from their elements (enzymes and substrates), and the development of the idea that biological form and function are based upon physicochemical dynamics.

Many biologically occurring compounds, for example, ethanol, sucrose, acetic acid, tartaric acid, and urea, were known in a relatively pure state before the advent of atomic theory in 1808. But, although organic chemistry was then "the chemistry of plant and animal substances or bodies which are formed under the influence of the vital force" (Berzelius, 1827), it was not possible to classify such substances (except grossly according to radicals, types and copulae) until the quantitative techniques initiated by Lavoisier, the analytical methods of Gay-Lussac, Thénard, Berzelius, and Liebig, and the valence theory of Frankland, Loschmidt, Kekulé, and Couper had given rise to the relatively simple but essentially modern structural theory which appeared in the decade 1850–1860. Then organic chemistry became the more general chemistry of the compounds of carbon (Kekulé, 1861), and it rapidly became evident that most of the substances which occur in the organisms comprising the phylogenetic scale fall into a few well-defined structural classes: fatty acids and lipids, amino acids and proteins, saccharides and polysaccharides, nucleotides and nucleic acids. The recognition that such natural structural classes exist was largely complete before the end of the nineteenth century, and was undoubtedly the chemical starting-point of systematic comparative biochemistry.

During the period that structural theory was developed and applied to biologically occurring substances, it was also recognized that life is associated with catalytic processes. Of course, alcoholic, putrefactive, and acetic fermentations were known since ancient times, but it was not until Pasteur's work that such processes were unequivocally connected with the metabolism of living organisms. In that same era, several "albuminous substances which possessed the power of producing catalytic reactions" (3) were discovered: amylase, emulsin, pepsin, and trypsin. Some controversy arose over the relationship between ferments which acted only as a part of living cells (organized ferments) and those which could act in the absence of such cells (unorganized ferments), called *enzymes* by Kühne (1878), but this controversy was resolved by Büchner's discovery that "zymase" could be separated from yeast cells. By 1901, Oppenheimer was able to define enzymes as "ferments produced by the living cell and adhering more or less firmly to them without having their activities bound up with the vital process as such" in spite of the fact that "we are still absolutely in the dark as to the chemical nature of ferments" (5). Furthermore—a fundamental

step in comparative biochemistry—"they can be detected everywhere in the organized world, so that we can regard them as constant, concomitant phenomena of life." Enzymes were found in bacteria, in yeasts, molds, in the sap and organs of higher plants, in insects and lower animals, in fishes, amphibia, and mammals. And, more important, "of all the multitudinous chemical processes in the living cell on which life depends, there is scarcely one which is not due to enzyme catalysis; there can be no life without enzymes" (*6*). This is a comparative generalization of transcendant importance because it describes one of the central unities of life. Such a generalization cannot be absolute because only a small proportion of the approximately two million living species has been investigated from this point of view, but it is obvious that, if plentiful examples can be drawn from every level of the phylogenetic scale and if no exceptions can be discovered, the probability that it is true for life in general is very high. This is also true of other comparative generalizations. On the other hand, the extent of biochemical diversity, which is very interesting in its own right, can only be determined by study of all living organisms. Nevertheless, there may be principles which govern its kinds and extent; these principles may well be discovered before the whole range of living species has been investigated.

Another major biochemical unity which became apparent in the nineteenth century concerned the biological conservation of energy. The principle of energy conservation, the first law of thermodynamics, had been developed by the work of Benjamin Thompson, J. R. Mayer, and von Helmholtz (1847), who extended the idea to biology. Evidence that the principle was applicable to living organisms was subsequently obtained by Rubner (1885), and Atwater and Bryant (1899), and provided experimental basis for the belief that the same laws which describe the transformations of energy in inanimate matter also describe them in living organisms.

Thus, the evolution of nineteenth century organic and physical chemistry provided the groundwork for major generalizations about biochemical unity. During the same period, some interest in the comparative aspects of biochemistry as such was also manifest, not only with respect to the structural composition, distribution and metabolism of the chemical components of living organisms, but also in regard to the relationship of these characteristics to the physiological, genetic, evolutionary, and ecological properties of life. In 1886, Krukenberg devoted attention to comparative biochemistry in his "Vergleichend-physiologische Vorträge," and Griffiths, in 1892, wrote a whole volume on the subject, entitled "Physiology of Invertebrata." This, unfortunately,

was largely a collection of errors, probably attributable to a lack of technical knowledge. In 1903, following a suggestion of Anton Dohrn, Otto von Fürth published his "Vergleichende chemische Physiologie der niederen Tiere," in which he endeavored to collect and systematize the biochemical knowledge of invertebrates. His classification was based upon the relationship of composition and function, as shown by the chapter titles: blood, respiration, nutrition, excretion, muscle, support substances, tissue pigments, reserve substances and ash constituents, products of sexual glands, and chemical relationships with the environment. Under each heading, the different phyla were considered. The same point of view is found in E. Baldwin's "Introduction to Comparative Biochemistry," published first in 1937, a third edition of which appeared in 1949. Baldwin writes in his preface: "The task of the biochemist is, after all, to study the physico-chemical processes associated with the manifestations of what we call life—not the life of some particular animal or group of animals, but life in its more general sense. From this point of view, a starfish or an earthworm, neither of which has any clinical or economic importance *per se*, is as important as any other living organism and fully entitled to the same consideration, and unless such forms do receive considerably more attention than is accorded to them at present, biochemistry, as yet hardly out of its cradle, will assuredly develop into a monster." In addition to his emphasis on the unitarian aspects of comparative biochemistry, Baldwin gives attention to biochemical diversity according to the physiological viewpoint already seen in von Furth's treatise.

One of us (M. F.) adopted a different point of view in his "L'evolution biochimique" (1944), trying to associate homologies and analogies in a study of biochemical unity and diversity. The author collected a number of examples of biochemical adaptations on the one hand, and on the other hand, of biochemical novelties among taxonomic divisions.

In "The Microbe's Contribution to Biology" (7), A. J. Kluyver and C. B. van Niel have compared systems of molecular dynamics within microorganisms with those in other forms of life. They show that the principles which appear to describe metabolism, mutation, and adaptation in microorganisms may apply to all forms of life.

III. The Scope of Comparative Biochemistry

Thus, the field of comparative biochemistry developed from roots in organic and physical chemistry, as well as in the biological sciences. What then is its scope?

At its most fundamental level, comparative biochemistry seeks to

compare the chemistry of life with the chemistry of the world (or worlds!) in which life occurs, and to discover the manifestations of matter and energy which characterize the degrees of life. At the molecular level, comparative biochemistry seeks chemical similarities and difference of composition and reaction throughout the phylogenetic scale, and compares the physicochemical mechanisms by which energy is transformed in organisms. As regards enzymes, Dixon and Webb "use the term 'comparative enzyme biochemistry' to include two distinct fields of study, namely, on the one hand comparison of the enzymic equipment of different organs and species, and on the other hand the comparison of pure preparations of a given enzyme obtained from different sources" (*6*). At higher levels of organization, comparative biochemistry seeks similarities and differences in those systems of reactions upon which biological form and function ultimately depend.

In endeavoring to achieve these objectives, comparative biochemistry has already developed a conceptual framework in which life is considered a phenomenon consistent with the inanimate phenomena of the universe. Within this framework, purely biological taxonomies and classifications of all aspects of life are clarified by a more fundamental physicochemical classification in which organisms are described in terms of molecular dynamics, and the differences between them are characterized as specific and secondary variations upon the essential ground plan shared by all living forms. The processes of evolution, growth, differentiation, mutation, and inheritance, common to all life, are understood as aspects of biochemical unity, and there is good reason to believe that the principles which govern the extent of possible diversity to which these processes lead will be worked out. And by discovering that chemistry which is essential to all forms of life, comparative biochemistry may provide decisive clues to the origin of life itself.

References

1. J. Baker, The cell theory: a restatement, history and critique. *Quart. J. Microscop. Sci.* **89**, 103–125 (1948).
2. E. Baldwin, "Comparative Biochemistry." Cambridge Univ. Press, London and New York, 1949.
3. M. Florkin, "L'Evolution biochimique." Masson, Paris, and Desoer, Liège, 1944.
4. L. J. Henderson, "The Fitness of the Environment," p. 312. Macmillan, New York, 1913.
5. C. Oppenheimer, "Ferments and Their Actions," p. 81. Griffin, London, 1901. (Translated by C. A. Mitchell.)
6. M. Dixon and E. C. Webb, "Enzymes," p. 621. Academic Press, New York, 1958.
7. A. J. Kluyver and C. B. van Niel, "The Microbe's Contribution to Biology." Harvard Univ. Press, Cambridge, Massachusetts, 1956.

CHAPTER 2

Thermodynamics of Living Systems

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I. Introduction

A. Purpose of the Chapter

Biological systems, as well as the rest of the universe, are continually undergoing physical and chemical changes. All such changes involve the gain or loss of energy, or the transformation of energy from one form into another. The purpose of this chapter is to establish the basic background necessary for an understanding of the thermodynamics or energy relations of biological systems.

Comparative biochemistry exemplifies the basic similarity of molecular structure and chemical reaction patterns in cells of all organisms. It is important to recognize a sort of "comparative biophysical chemistry" which may be regarded as being even more fundamental, since a consideration of thermodynamics and reaction rate theory provide a common basis for understanding not only life processes but the nature of the inanimate portion of the universe as well.

B. Scope of the Presentation

This chapter will be divided into three sections: (*a*) a brief development and discussion of classical thermodynamics with particular emphasis on the underlying assumption of equilibrium conditions; (*b*) an outline of concepts pertaining to steady state systems which are of particular interest to living organisms; and (*c*) absolute reaction rate theory as a complementary approach to the study of living systems.

II. Classical Thermodynamics

A. Introduction

One becomes acutely aware of the complex nature of living organisms when attempting to apply quantitative ideas to such systems. In this chapter we will be principally concerned with the reactions occurring on a cellular level, since this represents the fundamental structural and functional unit of organisms.

We tend to think of cells as microscopic chemical machines which carry on a multitude of activities. They consist of a complex solution throughout which are dispersed many solids and gases. The entirety is enclosed by a phase boundary through which some components are free to move while others are not. The nondiffusable elements, then,

are in a sense a permanent part of the interior of the cell, except as they too enter into reactions and disappear.

If one considers the cell as a system enclosed within another system, e.g., the environment in which the cell is embedded, it is obvious that there is a continual change of matter across the phase boundary or membrane. Materials enter, react, and waste products are extruded. Elaborate pathways have evolved by which energy is accumulated, transferred, stored, and released as the requirements of the cell demand. Comparative biochemistry reveals the universality of certain of these pathways and one sees variations of a central theme throughout all nature. Besides the movement of matter necessary to sustain life, there is a need for an energy flux as well. Heat produced by chemical reactions must be utilized within the cell, dissipated, or directed to energize reactions which are energy-deficient.

In addition to these properties most cells also possess the ability to duplicate themselves. Furthermore, they house an elaborate information center, capable of transmitting information from daughter cell to daughter cell with a remarkable degree of accuracy.

All of the above processes are carried out under very mild conditions, e.g., at room temperature and at a pH of about 7. All of this points to the marked difference of such systems compared with the usual models employed to explain classical thermodynamics. Classical theory concerns itself with equilibrium states and reversible reactions. While it is true that it takes cognizance of nonequilibrium states, it leads only to inequalities not sufficiently restricted to yield useful results. The steady state provides a fruitful alternative restriction.

Thermodynamics of irreversible processes is largely concerned with entropy production and nonequilibrium states. As we shall later see, such an analysis leads to a method of determining which processes become possible by means of an entropy coupling, e.g., the use of entropy production of another process. Since entropy does play the central role in classical as well as in irreversible thermodynamics, a great deal of attention will be devoted to it.

B. Equilibrium and Open Systems Defined; Steady States

It is more or less intuitive that a system left to itself will eventually come to equilibrium. After all backward reactions balance forward reactions, after all thermal gradients have disappeared, and after all pressure gradients have vanished, the system no longer changes with time. This time-independent state of an isolated system is called equilibrium. It is also possible to have another type of time-independent state which is quite different from the one above. Suppose that energy

and matter are entering, reacting, and leaving the cell at the same rate. This too is a time-independent state. Furthermore it more closely approximates the conditions existing in biological systems. Let us consider such a system composed of molecules and ions. These particles may exist in various energy states depending upon whether they are free or have formed chemical combinations. Let N_i represent the number of particles in a particular energy state i. In general the collisions, chemical reactions, and movement in force fields cause the individual elements to change energy states, and N_i may or may not change. We may express this idea in the form

$$\frac{dN_i}{dt} = \sum_j \left(\frac{dT_{ji}}{dt} - \frac{dT_{ij}}{dt} \right) \tag{1}$$

where dT_{ji}/dt denotes the number of elements entering state i from state j per unit time and dT_{ij}/dt denotes the number of elements leaving state i for state j per unit time. That is the change in state i is just the number of particles entering from all other states minus the number leaving it for any other state. If the right side of the equation does not vanish, N_i is a function of time and we have a nonstationary state. If the right side vanishes, N_i is not a function of time. If this is true for every i, the system is in a steady state. If this is true for every value of i and j, then

$$\frac{dT_{ij}}{dt} = \frac{dT_{ji}}{dt} \tag{2}$$

and the right side of the equation vanishes. But note that this is a much more restrictive imposition and corresponds to an equilibrium state. As an example consider a cell in which there are i components which may diffuse into or out of the cell. Then

$$\frac{dN_i}{dt} = T_i + P_i \tag{3}$$

$$= \left[\left(\frac{dN_i}{dt} \right)_{\text{chem}} - \left(\frac{dN'_i}{dt} \right)_{\text{chem}} \right] + \left[\left(\frac{dN_i}{dt} \right)_{\text{diff}} - \left(\frac{dN'_i}{dt} \right)_{\text{diff}} \right]$$

$$= \left[\left(\frac{dN_i}{dt} \right)_{\text{chem}} + \left(\frac{dN_i}{dt} \right)_{\text{diff}} \right] - \left[\left(\frac{dN'_i}{dt} \right)_{\text{chem}} + \left(\frac{dN'_i}{dt} \right)_{\text{diff}} \right] \tag{4}$$

where

T_i = the net rate of production of the ith component,
P_i = the net rate of consumption of the ith component,

$\left(\frac{dN_i}{dt} \right)_{\text{chem}}$ = the rate of production of the ith component by chemical reaction,

$\left(\frac{dN'_i}{dt}\right)_{\text{chem}}$ = the rate of consumption of the ith component by chemical reaction,

$\left(\frac{dN_i}{dt}\right)_{\text{diff}}$ = the rate of the ith component diffusing in, and

$\left(\frac{dN'_i}{dt}\right)_{\text{diff}}$ = the rate of the ith component diffusing out.

The steady state is when the right side of the equation vanishes, e.g., when the net rate of production is equal to the net rate of consumption. Equilibrium requires

$$\left(\frac{dN_i}{dt}\right)_{\text{chem}} = \left(\frac{dN'_i}{dt}\right)_{\text{chem}} \tag{5}$$

and

$$\left(\frac{dN_i}{dt}\right)_{\text{diff}} = \left(\frac{dN'_i}{dt}\right)_{\text{diff}} \tag{6}$$

Furthermore, for every chemical reaction into which i enters there must be an equilibrium, e.g.,

$$\left(\frac{dN_{i1}}{dt}\right)_{\text{chem}} = \left(\frac{dN'_{i1}}{dt}\right)_{\text{chem}} \tag{7a}$$

$$\left(\frac{dN_{i2}}{dt}\right)_{\text{chem}} = \left(\frac{dN_{i2}}{dt}\right)_{\text{chem}} \tag{7b}$$

.
.
.

etc.

Clearly, such a state in organisms is attained only through death.

Another interesting property of Eq. 3 has been pointed out by von Bertalanffy (*1*). For the steady state

$$T_i + P_i = 0 \tag{8}$$

must hold for $t \neq 0$. If both members are linear in N_i and are independent of t, the solution of this equation will be in the form

$$N_i = N_{i1}(x,y,z) + N_{i2}(x,y,z,t) \tag{8a}$$

where N_{i2} reduces to zero for certain limiting conditions. Note that the initial conditions do not appear in the steady state. Thus the final state may be reached from different initial conditions and in different ways. Such a system is termed *equifinal* and is in contrast to most physical

systems where the final state is determined by the initial conditions. Equifinality has been used by many, including Driesch (*1a*), as the principal argument of vitalism. Driesch maintained that forces different from those of known physics and chemistry were responsible for governing certain natural processes by foresight of the goal to be attained. Such forces then give rise to the same final result, namely a typical organism from a whole germ, a half germ, two fused germs, or after translocation of cells in the embryos of certain organisms. In the light of the above analysis, equifinality arises as a consequence of the model chosen and in no way suggests extraphysical forces.

C. A Simple Model

At this point we should like to introduce a simple model which will be used extensively throughout this chapter. It was originally suggested by von Bertalanffy (*1*). Consider a typical cell within an organism. A cell is a heterogeneous system enclosed by a phase boundary. Within this system the chemical reactions characteristic of life are being carried out. We shall refer to the cell proper as the α-phase. The ground substance in which the cell is embedded will be referred to as the β-phase.

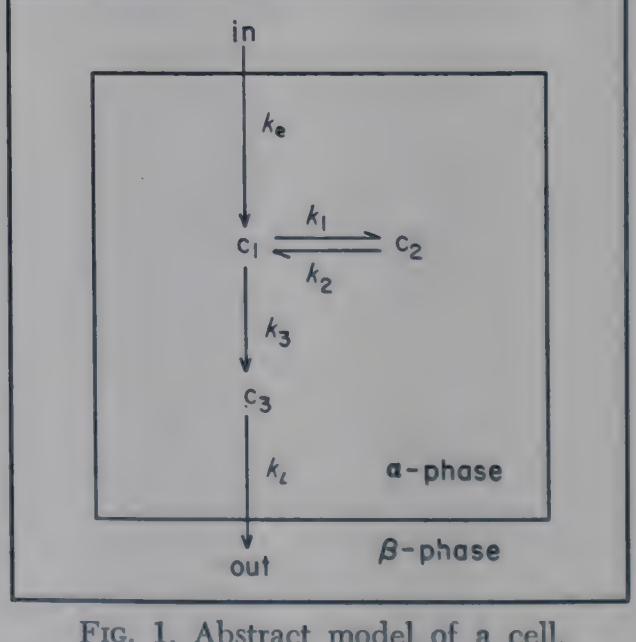


Fig. 1. Abstract model of a cell.

The two phases together are to be considered as being enclosed in an adiabatic jacket thus constituting an isolated system. In order to maintain the integrity of the cell, there must be a continual exchange of matter and energy across the phase boundary which we call the cell membrane.

Schematically, we may represent this model by the diagram in Fig. 1. We now assume that the transport of material c_1 into the cell is pro-

portional to the difference in the activity of c_1 on the outside and inside of the cell, $(A - a_1)$. Thus we may write

$$\frac{da_1}{dt} = k_e(A - a_1) - k_1a_1 + k_2a_2 - k_3a_1 \tag{9}$$

$$\frac{da_2}{dt} = k_1a_1 - k_2a_2 \tag{10}$$

$$\frac{da_3}{dt} = k_3a_1 - k_La_3 \tag{11}$$

At steady state conditions

$$a_1:a_2:a_3 = 1:\frac{k_1}{k_2}:\frac{k_3}{k_L} \tag{12}$$

Furthermore

$$a_1 = \frac{k_eA}{k_e + k_3} \tag{13}$$

Three important facts are pointed out by von Bertalanffy (*1*). (*a*) The composition of the steady state system remains constant but is not dependent on equilibrium. (*b*) The steady state ratio of components is a function only of the system's constants and not on environmental conditions. (*c*) From Eq. 13 we see that if k_3 is increased by an increase of catabolism within the cell, then a_1 shows a decrease and the quantity $(A - a_1)$ increases. Since we have assumed that transport of c_1 into the cell is proportional to this quantity, it follows that the transport increases. Thus the system reacts in such a way as to countermand the stress imposed. We recognize this as Le Chatelier's principle. The important inference is that we are dealing with a stable system.

With this introduction to the type of systems to which we would like to extend thermodynamic analysis, we now turn our attention to entropy and its central role in the theme we wish to develop. The concept of entropy is conveniently introduced by a consideration of classical thermodynamics. Such an approach will also present for review some of the important ideas which find wide application in biological systems.

D. Forms of Energy

Energy is usually defined in elementary texts as something which has the capacity to do work. This definition is not too precise since all forms of energy cannot be converted into work with equal efficiency even under ideal conditions. The concepts of energy and work have

grown out of a long history of human experience with the world on the mechanical level. We tend to think of work as representing what is done when we move an object from one place to another or from one level to a higher level. Energy is that which is expended in carrying out such processes. Also, from common human experiences, we get some concept of the storage of energy. For example, if we roll a large rock to the top of a hill we know that the rock can roll down again spontaneously, e.g., without any further expenditure of energy on our part. In some way, part of the energy expended in getting the rock to the top of the hill is stored up by virtue of the higher position of the rock. We are also acquainted with energy storage in living systems. One example would be the storage of energy in "high-energy" phosphate bonds which when involved in the appropriate reactions are capable of delivering energy which the organism can utilize.

It is useful to consider the various forms of energy. We are all acquainted with the familiar *potential* and *kinetic energies* of mechanical systems. In addition, there are a number of other forms of energy of interest in biological systems, or of utility in calculating thermodynamic quantities. These include: (*a*) *pressure-volume energy,* the energy required or produced when a gas is compressed or permitted to expand; (*b*) *surface energy* which is involved when the area of a surface possessing surface tension is increased or decreased; (*c*) *electrical energy* which is involved when an electric current flows between a potential difference; and (*d*) *radiant energy.*

Energy is sometimes regarded as being composed of a *capacity factor* and an *intensity factor* with the total amount of energy being the product of these two factors. Capacity factors are also termed *extensive factors* since their values depend on the quantity of the system involved. Intensity factors are independent of the size of the system and are often called *intensive factors.* If we double the size of a particular system, the energy content will be doubled since the capacity factor will be doubled. The intensity factor, of course, will remain the same. In pressure-volume energy, volume is capacity and pressure is intensity; in electrical energy the quantity of charge (coulombs) is capacity and the potential difference (volts) is intensity; and in chemical potential energy moles of material is capacity and chemical potential is intensity. The intensity factor, not the total energy, determines the direction of movement of energy. We can say crudely that energy of a particular type "moves" spontaneously from a region or situation of high intensity to one of low intensity. This movement continues until intensity is the same in all parts of the system.

E. Work

Several different kinds of work may be distinguished. Ordinary *mechanical* work is equal to the product of the distance some mass is moved in the direction of the applied force and the force necessary to bring about the movement. If the force is expressed in dynes and the distance in centimeters, the work done will be in ergs.

The calculation of *electrical work* is somewhat more complicated. The maximum possible electrical work (at constant pressure and temperature) which can be obtained from an electrical cell operating under reversible conditions is given by the following equation:

$$W = nFE \tag{14}$$

where W is the work in joules, n is the valence change involved in the reaction of the particular electrical cell, F is the Faraday constant of electricity, and E is the voltage of the cell. It should be pointed out that pressure-volume work is involved in certain electrical cells in addition to electrical work, e.g., when the cell produces a gas during operation.

For the calculation of pressure-volume work, consider a system in which a perfect gas is allowed to expand against a pressure essentially equal to the pressure of the gas itself. The maximum possible work produced in such a system is given by the equations:

$$W = \int_{V_1}^{V_2} P dV = \int_{V_1}^{V_2} nRT \frac{dV}{V}$$
$$= nRT \ln \frac{V_2}{V_1} = nRT \ln \frac{P_1}{P_2} \tag{15}$$

where V_1 and P_1 represent the initial volume and pressure, V_2 and P_2 represent the final volume and pressure, and n, R, and T have the same meanings as in the perfect gas equation.

In living organisms energy can be used to "pump" material through membranes against concentration gradients. This type of work is usually termed *osmotic work* and is equal to the difference in the *chemical potential* of the compound or ion under consideration on the two sides of the membrane. The chemical potential represents a type of energy associated with each component of a solution or gas mixture. This energy can be thought of as representing the tendency of a component to "escape" from one phase to another. Gibbs pointed out an interesting analogy between chemical potential and potential functions of mechanics. In mechanics, potentials depend solely on the position of one

body with respect to one another; similarly, the chemical potential is determined solely by the thermodynamic state of the phase in question as characterized by its temperature, pressure, and composition. The symbol assigned to denote the chemical potential is μ.

The work W required to move a solute from a solution where it has a chemical potential of μ_1 to a solution where it has a chemical potential of μ_2 is

$$W = n(\mu_1 - \mu_2) \tag{16}$$

where n is the number of moles of solute transferred.

The chemical potential may be expressed in terms of concentration by the equations

$$\begin{aligned} \mu_1 &= \mu^0 + RT \ln \gamma_1 m_1 \\ \mu_2 &= \mu^0 + RT \ln \gamma_2 m_2 \end{aligned} \tag{17}$$

where μ^0 is the chemical potential when $\gamma m = 1$, m is the molal concentration of solute, and γ is the *activity coefficient*. The product γm is called the *activity* and is denoted by a. *Ideal solutions* are those in which there is no interaction between molecules. Real solutions always manifest some interaction and the activity coefficient is the quantity the concentration must be multiplied by in order to account for the apparent chemical reactivity of the solute. The activity, then, may be thought of as the effective concentration in terms of chemical reactivity. If we substitute Eq. 17 in Eq. 16 we obtain

$$W = nRT \ln \frac{a_1}{a_2} \tag{18}$$

where W is the osmotic work done in transferring n moles of solute from a solution of lower activity a_2 to one of higher activity a_1. In dilute solutions, concentrations may be substituted for activities without any significant change in the results.

One of the central biological problems is the extent to which cell microstructure demands modification of the above equations for ideal solutions. This problem will be deferred until after the theory of reaction rates has been presented.

A final type of work that is often listed as taking place in certain biological systems is *photochemical work* as in bioluminescence reactions where chemical work leads to the production of radiant energy.

F. Reversible and Irreversible Processes

Thermodynamics can in theory be applied only to the so-called *reversible processes* which can be reversed by an infinitesimal change in

the reaction conditions. A reversible reaction is one which proceeds in such a way that it is always essentially at equilibrium. The concept of reaction rate or time to reach equilibrium is not involved. As a matter of fact, we usually must postulate that our ideal reactions will take place infinitely slowly in order that they be reversible. As has been emphasized by several writers, the field of classical thermodynamics might have better been termed thermostatics. Thermodynamically reversible reactions or processes do not occur to any extent in our physical universe. The reversible process may be regarded as the limiting condition to which actual processes approach. Such limiting conditions can be defined precisely and calculations of work, heat, energy, etc. made which can then be applied with reasonable reliability to actual processes.

With respect to biological systems none of the over-all reactions are readily reversible. For example a typical living cell is maintained in a high-energy, nonequilibrium condition by a continuous inflow of energy in the form of light or in the form of oxidizable organic compounds synthesized using light energy. However, if one considers a specific enzymatic reaction within a cell, he may find the reversible reaction

$$E_n \rightleftharpoons E_d$$

occurring. It is only in death that the cell comes to equilibrium, and, moreover, death is an irreversible process.

We must recognize then that thermodynamics gives us only an approximation, although in many cases a very good approximation, when applied to biological systems. In an attempt to provide a closer approach, a well-known field termed *thermodynamics of irreversible processes* has been developed. This branch of thermodynamics owes much to Onsager's attempts to treat irreversible processes on a microscopic level analogous to classical theory. In a later section we shall consider this field in more detail.

G. The First Law of Thermodynamics: Conservation of Energy

The sum of mass and energy in any kind of change is always a constant. This is the First Law of Thermodynamics. It takes the familiar mathematical form of

$$dE = dQ + dW \tag{19}$$

where dE is the change in energy accompanying a thermodynamic change of state of a system. Such a change may be due to the performance of work on or by the system, e.g., dW, and also to the transfer of energy to or from the system in the form of heat, e.g., dQ. The work

term is general and includes all forms of work—mechanical, electrical, etc.; that is it represents the algebraic sum of all the work terms. It is taken to be positive if work is done by the system. Likewise dQ is the algebraic sum of all heat entering or leaving the system. It is taken to be positive if heat is absorbed by the system.

H. Applications of the First Law

In this section we summarize the most important results of the First Law for chemical and biological systems.

Writing the First Law in the form

$$Q = \Delta E + W \tag{20}$$

we assume the only work performed is mechanical work, e.g., pressure-volume work. For constant volume

$$Q_V = \Delta E \tag{21}$$

so that the heat absorbed at constant volume is just the increase in internal energy. At constant P, the expression becomes

$$Q_P = \Delta E + P\Delta V \tag{22}$$

Let

$$\Delta E = E_2 - E_1$$

where E_1 and E_2 are the energy values for the initial and final states; we can likewise write

$$\Delta V = V_2 - V_1$$

Thus Eq. 22 becomes

$$Q_P = (E_2 - E_1) + P(V_2 - V_1) = (E_2 + PV_2) - (E_1 + PV_1) \tag{23}$$

P and V are state functions; therefore $E + PV$ is also a state function. It is an extensive property called the *heat content* or *enthalpy*. It is given the symbol H. Hence

$$H = E + PV \tag{24}$$

At constant P, we can therefore write

$$Q_P = \Delta H \tag{25}$$

The equation for the heat of a chemical reaction is

$$Q_P = \sum H \text{ (products)} - \sum H \text{ (reactants)} = \Delta H$$

A chemical reaction which liberates heat is termed *exothermic* while one which absorbs heat from the surroundings is termed *endothermic*.

By analogy with Q, ΔH is considered negative when heat is lost to the surroundings and positive when heat is gained by the system. The mistaken idea that the sign of ΔH for a chemical reaction determines whether or not the reaction proceeds spontaneously was once generally accepted. As we will show below, the sign of another thermodynamic parameter, ΔF (the change in *free energy*), is the determining factor in ordinary chemical and biological processes.

Thermochemistry, or the conversion of chemical energy into heat energy, is the direct application of the First Law to chemical reactions. Animal calorimetry is also a direct application of the First Law. The proof of the applicability of the First Law to animal metabolism consists in comparing the heat of oxidation of nutrients within and outside the body. Outside the body, ΔH is determined by an ordinary calorimeter. For example, if we burn glucose in a calorimeter we have

$$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O$$
$$\Delta H = -673 \text{ kcal.}$$

Within the body, the nature of the food oxidized is determined by measuring the respiratory quotient and urinary nitrogen. Quantitatively the amount of each substance (fat, carbohydrate, protein) is computed from carbon dioxide production, oxygen consumption, and urinary nitrogen excretion. Calorimetry is used to determine the heat dissipated by the animal and is compared with the heat as computed from the foods oxidized. Since the observed and computed heats usually agree within experimental error, this is taken as proof that the First Law does apply to animal processes.

It is not necessary to determine ΔH experimentally for many reactions since the data are available in the literature. It is also possible to calculate ΔH for many other reactions from the published values for the *heats of formation* of compounds from their elements, since for any chemical reaction, ΔH equals the sum of heats of formation of products minus the sum of heats of formation of reactants. Thus ΔH, as stressed above, is independent of the path and depends only on the reactants and their states and the products and their states. For example, let us calculate ΔH for the oxidation of glucose at 25° according to the following equation

$$C_6H_{12}O_6(\text{solid}) + 6O_2\ (\text{gas}) \rightarrow 6CO_2\ (\text{gas}) + 6H_2O\ (\text{liquid})$$

The heats of formation per mole are —301 kcal. for glucose, zero for oxygen (heats of formation of all elements are arbitrarily taken as zero), —94 kcal. for carbon dioxide and —68.3 kcal. for water. Thus ΔH for the over-all reaction is

$$\Delta H = [(6 \times -94) + (6 \times -68.3)] - [-301 + 0] = [-974] - [-301] = -673 \text{ kcal.}$$

which is the same value as obtained experimentally from the burning of glucose in a calorimeter.

Another technique, which is used in many cases where the ΔH for a reaction is difficult to measure directly, involves adding or subtracting an appropriate series of reactions, each of which has a known ΔH. For example, it is difficult to measure directly the ΔH for the burning of carbon in oxygen with the formation of carbon monoxide. It is fairly simple, however, to measure ΔH for the oxidation of carbon to carbon dioxide and the ΔH for the oxidation of carbon monoxide to carbon dioxide. These latter reactions can be subtracted algebraically to give the first reaction as follows:

$C\ (solid) + O_2\ (gas) \rightarrow CO_2\ (gas)$	$\Delta H = -94$ kcal.
$CO_2\ (gas) \rightarrow CO\ (gas) + \frac{1}{2}O_2\ (gas)$	$\Delta H = 67.6$ kcal.
$C\ (solid) + \frac{1}{2}O_2\ (gas) \rightarrow CO\ (gas)$	$\Delta H = -26.4$ kcal.

This technique for calculating ΔH indirectly can be applied to any combination of balanced equations which will add up algebraically to give the desired over-all chemical reaction.

I. The Second Law of Thermodynamics: Entropy

1. *Introduction*

The Second Law of Thermodynamics does not lend itself to such an intuitive grasp as does the First Law. Depending upon the problem at hand, it may be stated in numerous ways. We shall consider only those aspects pertinent to the problems of living systems.

The essential content of the Second Law is that all systems *left to themselves* manifest a drift towards equilibrium. We shall defer until later a more precise formulation, following a discussion of some of the concepts involved. The idea that natural processes tend towards equilibrium is inherent in the diffusion of material from a more concentrated solution to a less concentrated solution; in the flow of heat from hot to cold bodies; in the running down of a clock, etc. The final attainment of equilibrium may be very slow but it is inexorable. The capacity for spontaneous change is measured by how far away the system is from equilibrium. While the Second Law is sometimes referred to as the degradation of energy, this may be misleading and it would be better to follow the suggestion of Lewis and Randall (2) and speak of the "degradation of the system as a whole" in its ability to provide energy for external purposes.

In the discussion to follow, extensive use will be made of the concepts "irreversible" and "reversible." An irreversible process is one in

which it is impossible to restore all systems involved to their exact original states without adding energy from the outside. It is apparent, therefore, that all real processes are irreversible. However, one can imagine systems in which all restraints such as friction, for example, are infinitely small, and which permit with appropriate ingenuity a restoration of the systems concerned to the exact initial states. Such a hypothetical process is termed reversible and represents the limit of actual processes.

Inherent in the comparison of irreversible processes with reversible ones is the idea that systems may differ in degree of reversibility. One of the most fruitful contributions to modern science was the establishment of a quantitative measure of this difference. The measure of irreversibility is called the *increase in entropy* for a given process and is defined as

$$S_B - S_A = Q/T \tag{26}$$

where S_B is the entropy of the final state, S_A the entropy of the initial state, and Q is the amount of heat reversibly exchanged from one part of the universe to another part at temperature T.

Equation 26 implies that entropy is a state function since it depends only on the final and initial states. Thus, we speak of it as a property. In common with energy and heat capacity, we shall be mostly concerned with the difference between two states rather than with absolute values of the individual states.

As an example of the calculation of entropy change, consider the case of a perfect gas. The gas is introduced into a container separated from another identical one with a stopcock. The second container is evacuated. Let the volume of the container holding the gas be V_A and let the total volume of the containers after the stopcock is opened be V_B. Upon opening the stopcock the gas expands into the evacuated cylinder. We assume the containers to be an isolated system so that there is no change in energy and thus the temperature remains constant. Under such conditions if we compress the gas back into the original container by means of a frictionless piston, work is required with a concomitant surrender of an equivalent amount of heat to the environment. Assuming the compression to take place very slowly so that the internal pressure is kept equal to the external pressure, the amount of work done is

$$W = Q$$

or, for the case above,

$$W = Q = \int_{V_A}^{V_B} P dV \tag{27}$$

Now

$$PV = nRT \qquad \text{or} \qquad P = nRT/V \tag{28}$$

and therefore

$$Q = W = nRT \int_{V_A}^{V_B} \frac{dV}{V} = nRT \ln \frac{V_B}{V_A} \tag{29}$$

But Q/T is the increase in entropy of the free expansion; thus

$$S_B - S_A = nR \ln \frac{V_B}{V_A} \tag{30}$$

From this equation we deduce that entropy is an extensive property and the total entropy of a system is the sum of the entropies of its parts. Thus for an infinitesimal reversible process,

$$\sum_i dS_i = 0 \tag{31}$$

while for an irreversible process

$$\sum_i dS_i > 0 \tag{32}$$

The first expression forms the basis of classical thermodynamics, while the second expression introduces the thermodynamics of irreversible processes about which more will be said later.

One more consideration involves entropy as the measure of the randomness or lack of orderliness of a system. The increase in orderliness is associated with a decrease in entropy. The Second Law in terms of these concepts takes the following form: all real processes are accompanied by an increase of entropy.

Suppose we have two flasks of volumes V_A and V_B, respectively, which are connected through a stopcock. One mole of gas is introduced in V_A and V_B is evacuated. The stopcock is opened allowing the gas to expand into the evacuated container. The probability of finding all the molecules in one flask is, for flask A,

$$P_A = \left(\frac{V_A}{V_A + V_B}\right)^N$$

or, for flask B,

$$P_B = \left(\frac{V_B}{V_A + V_B}\right)^N$$

Thus

$$\frac{P_B}{P_A} = \left(\frac{V_B}{V_A}\right)^N$$

The probability that m molecules will be in V_A and $(N - m)$ molecules will be in V_B is

$$P_{m,N-m} = \frac{N!}{m!(N-m)!} \cdot \left(\frac{V_A}{V}\right)^m \left(\frac{V_B}{V}\right)^{N-m} = \frac{N!}{m!(N-m)!} \left(\frac{V_A}{V_B}\right)^m \left(\frac{V_B}{V}\right)^N \tag{33}$$

where V is the total volume, e.g., $V_A + V_B$. This probability has a maximum for

$$\begin{aligned} m &= \frac{V_A}{V} \cdot N \\ n &= \frac{V_B}{V} \cdot N \end{aligned} \tag{34}$$

This can be shown in the following manner. Taking the ln of both sides of Eq. 33 gives

$$\ln P = \ln N! - \ln m! - \ln (N - m)! + m \ln \frac{V_A}{V_B} + N \ln \frac{V_B}{V} \tag{35}$$

Applying Sterling's approximation in the form

$$\ln x! = x \ln x - x$$

differentiating with respect to m, and setting the result equal to zero gives

$$\frac{m}{N-m} = \frac{V_A}{V_B} \quad \text{or} \quad m = \frac{V_A}{V} \quad \text{and} \quad N - m = \frac{V_B}{V}$$

The most probable distribution is the one in which the number of molecules in each vessel is proportional to its volume.

In the case where $V_A = V_B = \frac{1}{2}V$, just as the stopcock is opened

$$P_A = (\tfrac{1}{2})^N \tag{36}$$

After a short time, the most probable distribution would be

$$P_{(N/2),(N/2)} = \frac{N!}{(N/2)!(N/2)!} \cdot (\tfrac{1}{2})^N \tag{37}$$

For large N, Eq. 37 is much greater than Eq. 36.

Thus we see that the gas passes from an initial state of low probability to a final state of high probability. The question then arises whether or not there is any basis for the parallelism between the increased probability of the final state and the increase in entropy which accompanies such spontaneous processes.

It turns out that there is, indeed, a relation between entropy and

the probability of a given state. Boltzmann's fundamental equation expresses this relation:

$$S = R \ln P \tag{38}$$

A state in which the molecules are assigned to definite locations is highly improbable and would be characterized by low entropy. We may, therefore, regard entropy as a direct measure of the randomness of a system.

We are now prepared to make a precise statement of the Second Law in the most general terms: "Every system, left to itself, will, on the average, change toward a condition of maximum probability" (*2*).

All real processes are accompanied by an increase in entropy. The extent of increase depends upon the energy and volume of the system. This can be seen most simply from the following argument: the change in entropy for a process is defined as

$$dS \geqslant dQ/T \tag{39}$$

where the inequality sign refers to a real process. From the First law, e.g.,

$$dE = dQ + dW$$

one sees that

$$\frac{dQ}{T} = dS \geqslant \frac{dE + dW}{T}$$

or

$$T\,dS \geqslant dE + dW \tag{40}$$

If for a given process E is constant, then $dE = 0$. If no work is done against an external pressure, then $dW = 0$. A sufficient condition for dW to equal zero is for V to be constant. Writing these conditions as subscripts, we have

$$(dS)_{E,V} \geqslant 0 \tag{41}$$

Thus it is that the entropy of a system increases spontaneously until it is a maximum consistent with the energy and volume of the system. Stated in other words, the entropy of a system at equilibrium is a maximum at constant energy and volume. This obviously provides a criterion for equilibrium.

2. *Statistical Nature of the Second Law*

The Second Law considered from a molecular-kinetic point of view is a statistical law. It expresses the tendency toward randomness or disorder in a system which consists of a large number of particles. As an

example, consider the case of diffusion. Let us suppose we have a container which is divided into a large section filled with water and a smaller section to the left filled with a dye solution. A partition separates the two sections thus preventing the two liquids from mixing. Suppose now that the partition is removed. After a sufficient time interval, one observes that the solution has become uniformly colored.

Diffusion does not necessarily arise as a result of interaction between molecules such that the dye molecules are forced away from a crowded region. To a first approximation, every dye molecule behaves independently of the others. Diffusion is a statistical phenomenon. Following the example of Schrödinger (*3*), if the container is imagined to be cut up into thin slices we have the following situation: at the time when the partition is lifted out at the left side there is a great concentration of dye molecules. Each molecule is being jostled by its neighbors until it acquires sufficient energy to allow it to jump into an empty site or "hole." Now the probability that the molecule will jump to the left is just the same as that it will jump to the right, but at the instantaneous border which exists just as the partition is removed, more dye molecules are in the thin slice to the left. Thus, more dye molecules will jump to the right. This process will continue until there is a uniform distribution of the dye molecules throughout the solvent. Now the Second Law states that in such a system there will be a drift toward the most random state, which in this case results after the dye molecules have distributed themselves equally throughout the solution.

If we consider one individual dye molecule, we see that the probability of its jumping in any direction is the same. Thus, the Second Law applied to an individual molecule has no meaning, for in this case any distinction between disordered energy, i.e., heat, and ordered energy, i.e., work, disappears.

Now suppose we insert the partition back into its original position. Furthermore, suppose we equip the partition with a molecular gate which is attended by a Maxwell demon. This creature, invented by the famous physicist, Clerk Maxwell, has the remarkable faculty of being able to discriminate between solvent molecules and dye molecules. The demon operates the gate in such a manner as to permit only colored molecules to pass to the left while solvent molecules are allowed to pass only to the right. After a while, one would find all the colored molecules completely separated from the solvent molecules and a return to the original condition results. This change, of course, is associated with a decrease in entropy. Helmholtz was the first to raise a serious scientific question along these lines. He inquired whether microorganisms may not have the ability of choice attributed to the hypothetical

Maxwell demon. If so, then it would be possible for systems to exist where such microorganisms could carry out chemical reactions in which the entropy as a whole, including the materials of the organism themselves, would decrease. Indeed, N. Wiener (*4*) in his "Cybernetics" presents the hypothesis that enzymes may well be metastable Maxwell demons. Further consideration of the problem, however, suggests that this is not likely.

3. *Life and the Second Law*

Significant differences exist between living and inanimate systems. The most elaborate inanimate structure, i.e., a perfect crystal, is infinitely inferior to a living cell in terms of its ability to reproduce itself, repair damage sustained by it, etc. Furthermore, through metabolic processes a cell maintains itself in a condition of nonequilibrium by the utilization of energy supplied from without. In temperature sensitivity there is also a vast difference. Crystals are most stable at absolute zero whereas cells are stable only within a very narrow temperature range. Cells are highly organized structures composed of many atoms which manifest a greater organization than their constituent atoms. Such orderliness is characterized by low entropy. Thus living cells appear to be creating "negative" entropy.

Living systems also are characterized by other hard-to-explain properties. Evolution always towards the more complex is puzzling. Perhaps one of the most intriguing aspects of life is the hereditary mechanism. We have seen that the Second Law is based on statistical considerations. Roughly, these laws are accurate to within a probable relative error of $1/\sqrt{n}$ where n is equal to the number of molecules that cooperate to bring about the law. However, in the hereditary mechanism incredibly small groups of atoms, which lie far *beyond* statistical treatment, are active agents in determining the cause of extremely orderly events within living systems. Schrödinger (*3*) compares the hereditary "code script," i.e., chromosomes, with the all-penetrating mind conceived by LaPlace. They are the law code and executive power. After reviewing the experimental evidence characteristic of the hereditary substance, Schrödinger concurs with Delbrück's model, i.e., that the gene is a macromolecule capable of isomeric changes which may lead to a vast number of rearrangements. On the basis of such a model, he concludes: "Living matter, while not eluding the laws of physics as established up to date is likely to involve other laws of physics hitherto unknown, which, however, once they have been revealed, will form just as integral a part of this science as the former."

Thus we come to the ultimate question of this section. What is the

position of life with respect to the Second Law? The Second Law means "death by confinement." This is what is meant by "left to itself" in the formal statement given in Section II,*I*,1.* Living systems avoid this death sentence since they are open systems and thus not confined. Stated in another way, all processes in nature show an inexorable increase in entropy on the over-all balance sheet. In the case of life, the approach towards maximum entropy, i.e., death, can only be postponed, not prevented, for exactly the length of a life time by continually consuming from the environment "negative entropy."

4. *Free Energy*

The idea of the drift towards maximum randomness provides a most fundamental way of viewing the Second Law, but there is also another measure which may be applied to living organisms which compares thermodynamically to chemical reactions carried out at constant temperature and pressure. In such a system, heat may flow to and from the environment. To get at this measure consider the definition of Gibbs free energy F:

$$F = E - TS + PV = H - TS \tag{42}$$

Hence

$$dF = dS - T\,dS - S\,dT + P\,dV + V\,dP$$

Subtracting

$$T\,dS \geqslant dE + dW$$

gives

$$dF \leqslant -S\,dT + V\,dP - (dW - P\,dV) \tag{43}$$

Thus for a change in dF at constant T and P

$$dF \leqslant -(dW - P\,dV) \tag{44}$$

From the above equation it is seen that the free energy is the maximum amount of mechanical energy obtainable from a process. Assuming that work is $P\,dV$ work only, then

$$dF \leqslant V\,dP - S\,dT \tag{45}$$

and

$$(dF)_{T,P} \leqslant 0 \tag{46}$$

Thus, in any real irreversible (spontaneous) process the free energy always decreases, providing that the only exchange with the surround-

* See p. 32.

ings is heat. Note that there is nothing explicit or implied about the rates of such spontaneous processes.

5. *Relation between the Free Energy and the Equilibrium Constant*

Consider the reaction

$$N_2 + 3H_2 \rightleftharpoons 2NH_3$$

At equilibrium, according to the law of mass action,

$$\frac{[NH_3]^2}{[N_2][H_2]^3} = K$$

In other words, there is a definite ratio of products to reactants. What determines this ratio?

Consider a reaction chamber from which there protrudes three evacuated cylinders. In the chamber a mixture of hydrogen, nitrogen, and ammonia are in equilibrium so that the above reaction obtains. The free energy change when the reactants, each at one atmosphere pressure, are combined to form NH_3 is calculated as follows: 1 mole of N_2 at one atmosphere contained in an independent cylinder is reversibly introduced into the first evacuated cylinder without expansion of the gas. In the process an amount of work, $PV = RT$, is done by the atmosphere. Similarly 3 moles of H_2 are introduced into the second evacuated cylinder resulting in work, $3RT$, being done on the H_2. Next, the nitrogen is reversibly expanded (or compressed) to pressure p_1, its equilibrium partial pressure in the reaction chamber. The work done by the gas is

$$\int_{P=1}^{p} P\,dV = RT \ln (1/p_1) \tag{47}$$

Likewise the expansion of H_2 from one atmosphere to p_2 yields an amount of work $3RT \ln (1/p_2)$. Now we simultaneously introduce the 1 mole of N_2 and the 3 moles of H_2 reversibly into the reaction chamber and extract 2 moles of NH_3 into the third evacuated cylinder. In each case this is done through a semipermeable membrane which allows only the appropriate gas to pass between the cylinders and the reaction chamber. In this process an amount of work, $p_1V_1 = RT$, must be done on the N_2; and amount $3RT$ on the H_2; and the work done by the NH_3 is $2RT$. If the NH_3 is now expanded (or compressed) from its equilibrium pressure, p_3, to one atmosphere and taken from the vacuum reversibly into the air, the work, $2RT \ln (p_3/1)$, is done in the expansion and the work, $2RT$, must be done against the atmosphere. All these work terms added together give the change in free energy:

$$\Delta F^\circ = -RT \ln \frac{p_3^2}{p_1 p_2^3} \tag{48}$$

or

$$\frac{p_3^2}{p_1 p_2^3} = e^{-\Delta F^\circ/RT} = K \tag{49}$$

where the superscript $^\circ$ on ΔF° indicates that we started with the reactants in a standard state, usually taken to be one atmosphere, and ended with the products in the same state. Thus, we see that the ratio of the products to the reactants is determined by the change in free energy and temperature alone. It should also be noted that the maximum amount of useful work obtainable from the reaction is determined from the change in free energy.

For the general equilibrium since

$$\sum_i n_i A_i \rightleftharpoons \sum_f n_f A_f$$

we have

$$\Delta F^\circ = -RT \ln \left(\prod \alpha_f^{n_f} / \prod \alpha_i^{n_i} \right) = -RT \ln K \tag{50}$$

Here in each case n_i represents the number of reactant molecules A_i combining to give n_f molecules of product A_f. The symbols Σ and Π represent, as usual, the sum and products, respectively, over the various quantities following the symbol. The α's in each case are the equilibrium activities. The expression for the change in free energy for *any arbitrary activities* at constant T and P is

$$\Delta F = \Delta F^\circ + RT \ln \left(\prod \alpha_f^{n_f} / \prod \alpha_i^{n_i} \right) \tag{51}$$

where ΔF° is defined by Eq. 50 and the α's now represent any arbitrary activities.

For reactions in solution, Eq. 51 still gives the free energy change for a process where the reactants at unit activity are changed over into products at unit activity. In this case it is convenient to assume the activities equal to the concentrations in dilute solution because in the low concentration range the work done by n moles of a substance in going from concentration c_i to c_j is $nRT \ln c_i/c_j$ and at any concentration this work is equal to

$$nRT \ln \alpha_i/\alpha_j = nRT \ln \gamma_i c_i/\gamma_j c_j \tag{52}$$

We thus have a general equation relating the standard free energy change for a reaction to the activities of the participating substances in their equilibrium state.

Rewriting Eq. 49 we have:

$$K = e^{-\Delta F^\circ/RT} = e^{-(\Delta H^\circ - T\Delta S^\circ)/RT} \tag{53}$$

When Eq. 53 is applied to liquids, it is of interest to calculate the effect on the equilibrium constant of changing pressure from one atmosphere to a pressure p. Since

$$\frac{\partial F}{\partial P} = V \tag{54}$$

it follows that

$$\frac{\partial \Delta F^\circ}{\partial P} = \frac{\partial F_f}{\partial P} - \frac{\partial F_i}{\partial P} = V_f - V_i = \Delta V \tag{55}$$

Here the subscripts f and i refer to the final and initial states, respectively, where

$$\begin{aligned} \Delta F_p^\circ &= \Delta F_1^\circ + \int_{p=1}^{p} \Delta V \, dp = \Delta F_{p=1}^\circ + \overline{\Delta V} \int_1^p dp \\ &= \Delta F_{p=1}^\circ + \overline{\Delta V} P \end{aligned} \tag{56}$$

In Eq. 56 we have $P = p - 1$; and the subscripts on the ΔF's now indicate the hydrostatic pressure. Thus, for reactions in solution, we can write

$$K = e^{-\Delta F_p^\circ/RT} = e^{-(\Delta F_1^\circ + P\overline{\Delta V})/RT} \tag{57}$$

The equilibrium constant is of universal importance in reactions. Its meaning is fundamentally the same, whether we are concerned with equilibrium in the usual thermodynamic sense, or whether we deal in the quasi equilibrium between the normal and activated states in rate processes, as will be discussed in Section IV,B of this chapter. Ordinarily, with a reaction in solution at atmospheric pressure, ΔV is not taken into account, as it is rarely large enough, i.e., not often more than a few cubic centimeters per mole, to make any difference in the value of ΔF_p°. Furthermore, with most reactions in solution, ΔV is so small that its influence becomes appreciable only at very high hydrostatic pressures of the order of several thousand atmospheres. In biological processes, however, which are likely to be under the control of large enzyme molecules, ΔV may be quite large, of the order of 100 cc. per mole. Reactions which proceed with such large volume changes are readily influenced by moderate hydrostatic pressures of a few hundred atmospheres, such as occur at depths in the sea. Thus, the influence of hydrostatic pressure on equilibria and reaction rates in biology becomes of distinct interest and of importance in analyzing the reaction mechanism.

From the practical point of view, Eq. 50, $\Delta F^\circ = -RT \ln K$, represents one of the most useful relationships developed in this section since it permits calculating the equilibrium constant for a reaction if ΔF° is known or ΔF° if the value of K can be determined. It must be remembered that ΔF° is the standard free energy change, i.e., the change in free energy in a reaction in which the reactants in their standard states (unit activity for solutions, one atmosphere for gases, etc.) react to form products in their standard states. For illustration we will take an example used by West (5). The following reaction, one of the steps in glycolysis, has an equilibrium constant at 38° of approximately 19.

$$\text{Glucose-1-phosphate} \rightleftharpoons \text{Glucose-6-phosphate}$$

Thus

$$\Delta F^\circ = -RT \ln K = -RT(2.303) \log 19 = -1800 \text{ cal.}$$

If the reactants and products are not at their equilibrium activities, as is usually the case in living systems, Eq. 51 can be used for calculating the actual free energy change in a reaction. For a reversible reaction $A + B \rightleftharpoons C + D$, Eq. 51 may be put in the simplified form

$$\Delta F = \Delta F^\circ + RT \ln \frac{[C][D]}{[A][B]} \tag{58}$$

where, in dilute solutions, $[C]$, $[D]$, etc. represent the concentrations of the products and reactants.

6. *The Relation Between ΔF° and Standard Oxidation Potentials*

There is one more biologically important result that will be developed, the relation between free energy changes and standard oxidation potentials.

Consider the general reaction

$$aA + bB + \ldots \rightleftharpoons lL + mM + \ldots$$

The free energy of a given mixture may be expressed in terms of chemical potentials by the following equations

$$\begin{aligned} F_{\text{reactants}} &= a\mu_A + b\mu_B + \ldots \\ F_{\text{products}} &= l\mu_L + m\mu_M + \ldots \end{aligned} \tag{59}$$

where we have assumed T, P, and the composition to be constant. The free energy change of the reaction is

$$\begin{aligned} \Delta F_{T,P} &= F_{\text{products}} - F_{\text{reactants}} \\ &= (l\mu_L + m\mu_M + \ldots) - (a\mu_A + b\mu_B + \ldots) \end{aligned} \tag{60}$$

We have previously stated that

$$\mu = \mu^\circ + RT \ln a \tag{17}$$

where μ° is the value of μ in the standard state, and a is the activity of the component under discussion. Substituting this value with the appropriate subscript into Eq. 60 gives

$$\begin{aligned}\Delta F_{T,P} &= l(\mu_L^\circ + RT \ln a_L) + m(\mu_M^\circ + RT \ln a_M) + \cdots \\ &\quad - (\mu_A^\circ + RT \ln a_A) - b(\mu_B^\circ + RT \ln a_B) - \cdots \\ &= l\mu_L^\circ + m\mu_M^\circ + \cdots - a\mu_A^\circ - b\mu_B^\circ - \cdots + RT \ln \frac{a_L{}^l \cdot a_M{}^m \cdots}{a_A{}^a \cdot a_B{}^b \cdots} \\ &= \Delta F_T^\circ + RT \ln \frac{a_L{}^l \cdot a_M{}^m \cdots}{a_A{}^a \cdot a_B{}^b \cdots}\end{aligned} \tag{61}$$

Now the product

$$\frac{a_L{}^l \cdot a_M{}^m \cdots}{a_A{}^a \cdot a_B{}^b \cdots}$$

is just the expression for the equilibrium constant, K. At equilibrium $\Delta F_{T,P} = 0$, hence

$$\Delta F_T^\circ = -RT \ln K$$

a result previously determined. In general, however, the activities may have any arbitrary values and the form of Eq. 61 is retained.

When the reactants form part of a galvanic cell, the change in free energy is just the electrical work done, i.e.,

$$-\Delta F = n\mathsf{F}E \tag{62}$$

where n is the number of electrons involved in the reaction, F is the Faraday constant, and E is the electrode potential. This equation is of importance since it permits the calculation of standard electrode potentials from free energy data. Replacing F and F° by $-n\mathsf{F}E$ and $-n\mathsf{F}E_0$, respectively, in Eq. 61 the result is

$$E = E_0 - \frac{RT}{n\mathsf{F}} \ln \frac{[a_L]^l[a_M]^m}{[a_A]^a[a_B]^b} \cdots \tag{63}$$

If concentrations are used in place of activities, an approximation which is often made, the equation becomes

$$E = E_0 - \frac{RT}{n\mathsf{F}} \ln \frac{[C_L]^l[C_M]^m}{[C_A]^a[C_B]^b} \cdots \tag{64}$$

Consider now a general redox reaction

$$\text{Reduced form} = \text{Oxidized form} + ne^-$$

Then

$$E = E_0 - \frac{RT}{nF} \ln \frac{[Ox]}{[Red]} \tag{65}$$

Many reactions in biological systems, especially those involved in energy transfer, are of the oxidation-reduction type. Thus Eq. 65 has practical applications since if E_0 is known for a system it is possible to calculate the actual redox potential, E, for any degree of oxidation of the system. Since E and F are related as shown in Eq. 62, E_0 is also a measure of the redox tendency of a system. Thus, if we assume oxidizing systems as the more positive as is usually done in biochemistry (a convention which is just the opposite to that used by American physical chemists), a system with an E_0 of $+0.3$ volts will oxidize a system with an E_0 of $+0.1$ volts, and will in turn be oxidized by a system with an E_0 of $+0.5$ volts. In the aerobic oxidation of β-hydroxybutyrate to acetoacetate in living material, the two electrons removed are transferred by several separate steps to oxygen with the formation of water. These steps, with their associated potentials, are shown in a provisional simplified form in Table I. The values of E_0' (E_0' is the

TABLE I
AN EXAMPLE OF A COUPLED REACTION[a]

Redox couple	E_0' (volts)
β-Hydroxybutyrate/Acetoacetate	-0.35
Reduced pyridine nucleotide (DPNH)/Oxidized pyridine nucleotide (DPN^+)	-0.32
Reduced flavoprotein ($FAD \cdot H_2$)/Oxidized flavoprotein (FAD)	-0.20
Reduced cytochrome c (Fe^{2+})/Oxidized cytochrome c (Fe^{3+})	$+0.25$
Reduced cytochrome oxidase (Fe^{2+})/Oxidized cytochrome oxidase (Fe^{3+})	$+0.55$
$H_2O/\frac{1}{2}O_2 + 2H^+ + 2e^-$	$+0.82$

[a] Table showing an example of the reaction chains of coupled redox reactions involved in the biological oxidation of organic compounds. Each redox couple with its indicated E_0' will oxidize the couples above it. In living cells the enzyme systems are apparently arranged such that the electrons removed from the initial compound are transferred in an orderly fashion down the sequence until they ultimately combine with molecular oxygen.

E_0 at the more physiological condition of pH 7 rather than at unit hydrogen ion activity) were taken from reference 6. The approximate ΔF for each step in the sequence may be calculated using Eq. 62 and the difference in the E_0' values of the two systems in the step. The over-all ΔF involved in transferring two electrons from β-hydroxybutyrate to oxygen by the reaction system above can also be calculated. The total potential difference through which the electrons move is

$0.82 + 0.35 = 1.17$ volts. Substituting this value into Eq. 62 we get

$$\Delta F = -nFE = -2 \times 96{,}500 \times 1.17 = -226{,}000 \text{ joules}$$
$$= -226{,}000/4.18 = -54{,}000 \text{ cal.} = -54 \text{ kcal.}$$

It must be recognized that the above calculation is only an approximation since the reactants are obviously not in their standard states. Also the reaction system is much more likely to be in a steady state condition rather than in equilibrium but this is not a significant complication.

J. Third Law

It is possible to determine the thermodynamic equilibrium constant for a reaction without requiring the measurement of a single equilibrium state of the reaction itself by resorting to the Third Law of Thermodynamics.

The Second Law fixes the entropy of any state provided it is known for some particular state, say the crystalline state at absolute zero. The Third Law provides a simple method for determining entropy changes of reactions since it gives us the information that ΔS for any reaction carried out for crystalline systems at absolute zero is zero. Consider, for example, the reaction $A + B \rightleftharpoons C + D$ at some temperature T°K. To calculate ΔS for the reaction the following scheme may be used. Suppose we reduce the temperature of the reactants to zero degrees and allow the reaction above to proceed. By the Third Law, ΔS for the reaction is zero. We next warm the products from 0°K to T°K. The process is shown in Fig. 2. The cooling of the reactants is

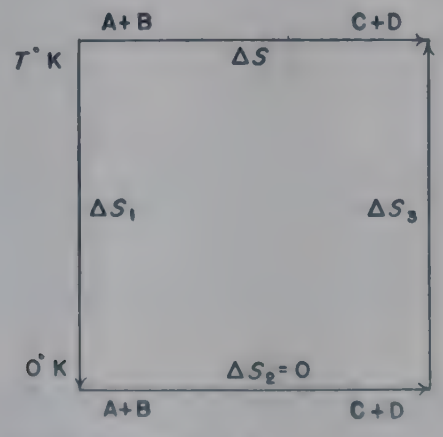


Fig. 2. Application of the Third Law to the calculation of ΔS.

accompanied by an entropy change ΔS_1, and the warming of the products involves an entropy change ΔS_3. These entropy changes can be determined experimentally. As a corollary that ΔS is a state function,

$$\Delta S_1 + \Delta S_3 + \Delta S = \Delta S_2 = 0$$

Thus if ΔS_1 and ΔS_3 are known, ΔS for the reaction at $T°K$ can be easily calculated. The importance of knowing ΔS for a reaction is, that if ΔH is determined, an experimentally feasible operation, the equation

$$\Delta S = \frac{\Delta H - \Delta F}{T} \tag{66}$$

provides a way of determining ΔF, and consequently the thermodynamic equilibrium constant without requiring the measurement of a single equilibrium state of the reaction itself.

In the pages to follow, ΔF will assume an important role in describing the energetics of living systems. If a particular process is characterized by a large positive ΔF, it cannot be expected to go spontaneously, but only if energy is added in an appropriate manner. If ΔF is small, either positive or negative, the reaction may be made to increase in one direction or the other by adjusting the ratio of the products to the reactants. If ΔF has a large negative value the process may be expected to go spontaneously to near completion. Once again, it is noted that there is nothing implied about the rate associated with a process characterized by a large negative ΔF.

A reaction which is accompanied by a decrease in free energy is said to be *exergonic*, while a reaction which proceeds with an increase in free energy is termed *endergonic*. One way of getting energy into an endergonic reaction would be to couple it with an exergonic one. The only thermodynamic requirement for an exergonic reaction to drive an endergonic one is that the exergonic reaction expend more free energy than is gained by the reaction to be driven, requiring, of course, that the entire process be exergonic. These types of reaction find great utility in biological systems. A more complete discussion of the theory of coupling of exergonic and exothermic reactions will be presented in a later section.

K. Energetics

1. *Introduction*

Living organisms, like any machine, require a constant source of energy to keep them functioning. In terms of free energy, we may say that some mechanism must be present to capture free energy and convert it into fuel which the organism may use. The major source of free energy for our world is from the nuclear reactions taking place in the sun. These reactions may be summarized by the equation

$$4_1H^1 \rightarrow {}_2He^4 + 2_1e^0 + h\nu$$

The term $h\nu$ represents very hard gamma radiation which is absorbed by electrons and re-emitted as smaller quanta. Eventually, after con-

siderable absorption and re-emission, the energy $h\nu$ is radiated from the surface of the sun as sunlight. The spectral distribution shows the maximum to be at 0.48μ. This radiation represents the large pool of free energy from which organisms must draw, directly or indirectly, in order to exist.

2. *Accumulation of Free Energy by Autotrophs*

The autotrophs are those organisms capable of drawing directly from the free energy pool to manufacture energy-rich compounds using CO_2 as the only source of carbon. This class includes all photosynthesizing plants. The over-all reaction for photosynthesis is sometimes depicted schematically as

$$CO_2 + H_2O + k\nu \rightarrow (CH_2O)_x + O_2$$

The modern trend, however, is to break the above equation into two parts in accordance with van Niels' (7) suggestion. Thus, the first reaction may be written

$$H_2O + k\nu \rightarrow [H] + [OH]$$

In green plants and other O_2-producing organisms, the oxidizing agent is disposed by conversion into molecular O_2:

$$2[OH] \rightarrow \tfrac{1}{2}O_2 + H_2O$$

The reducing agent [H] is used in the reduction of the CO_2 to produce ultimately all of the carbon compounds of the photoautotropic organisms. The second reaction may be depicted as

$$CO_2 + 4[H] \rightarrow (CH_2O)_x + H_2O$$

Intense work with radioisotopes has greatly clarified the actual pathway of carbon in green plants (8).

3. *Expenditure of Free Energy*

Only a small fraction of the total energy output of the sun is captured by living organisms. This energy is stored as chemical potential in carbon compounds for longer or shorter periods. Eventually, this chemical potential is released as free energy during exergonic chemical reactions, and a portion is utilized for the activities characteristic of life. It must be realized, of course, that the chemical potential of these carbon compounds may be used directly by the plant which produces them for its own designs, or that they may be ingested by the other large class of organisms, the heterotrophs. This class comprises those organisms which are incapable of manufacturing their own food, and therefore, depend upon the autotrophs.

In discussing the energetics of living systems, the first consideration to keep in mind is that they obey the First Law of Thermodynamics. It is convenient to categorize the multitude of reactions taking place within cells, e.g., metabolism, into two parts: (*a*) anabolism, which consists of the formation of complex molecules from simpler reactants, a process which requires energy; and (*b*) catabolism, a process which breaks larger molecules into smaller ones with a subsequent release of energy. An important corollary to the fact that living systems obey the First Law is that energy required in anabolic activities must be supplied by catabolic processes. This leads to the idea of coupling whereby the catabolic energy may be utilized for synthesis and other activities. In order to pursue this idea, let us first discuss the concept of reversibility in terms of biological reactions.

All chemical reactions tend to equilibrium if left to themselves, e.g.,

$$A + B + C + \cdots \rightleftharpoons L + M + N + \cdots$$

Thermodynamics has nothing to say about the rate of attainment of equilibrium; however, in the case in which a suitable catalyst is present equilibrium is rapidly established. In biological systems these catalysts are generally present in the form of enzymes. We have seen that the composition of the equilibrium mixture is determined by the over-all free energy change of the system and by the activities of the reactants and products. When we say a reaction is irreversible, e.g.,

$$A + B + C + \cdots \rightarrow L + M + N + \cdots$$

what we mean is that the free energy of the products is much smaller than the free energy of the reactants. Thus, the forward reaction greatly predominates over the backward reaction. The over-all reaction is therefore considered as irreversible in the forward direction. For example, in a biological system consider a catabolic process characterized by

$$A \rightarrow L + M + N + \cdots ; \qquad \Delta F = -Q \text{ kcal.}$$

Assuming the presence of an appropriate enzyme, Q kcal. per mole of free energy become available for anabolic activities or for other endergonic processes. In an *in vitro* preparation, where there is no suitable transformer to convert the free energy into an appropriate form for synthesis or biological work, the free energy is dissipated as heat in the amount given by

$$\Delta H = \Delta F + T\Delta S$$

In principle, to drive the reaction backward, e.g., to anabolize the products, Q kcal. of energy must be supplied. The Second Law assures

us that in order for heat to be converted into work it must operate between a hot source and a cold sink. In biological systems, the temperature is essentially constant; therefore reversal of the above reaction cannot be effected merely by the addition of heat. The principal mechanism of reforming the reactants evolved by living organisms is to alter favorably the free energy of one of the products. This is often done by incorporating into it a phosphate radical which will be represented in this discussion by *P*. A typical reaction of this type may be represented by the following scheme:

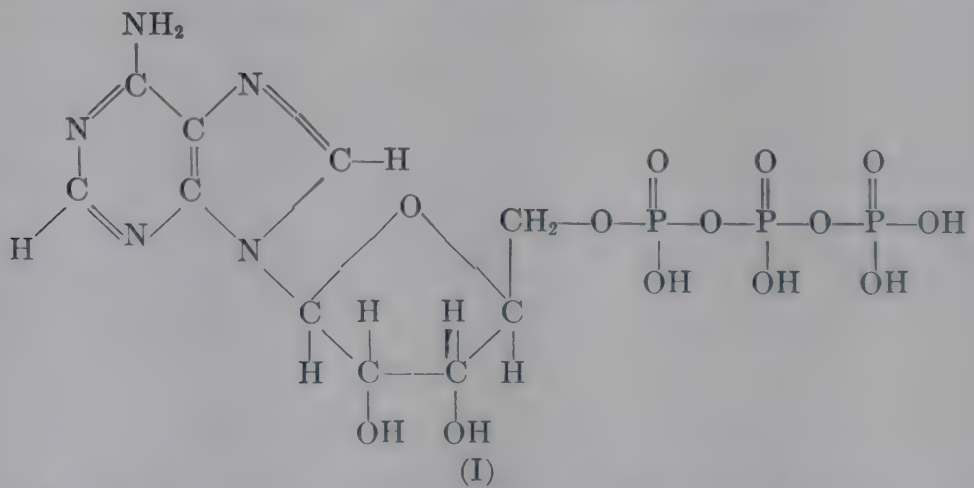
$$L + M + N + \cdots + X\text{-}P \rightarrow L\text{-}P + M + N \cdots + X \tag{67}$$

In the presence of a suitable enzyme the following reaction takes place:

$$X + L\text{-}P + M + N + \cdots \rightarrow A + B + C + \cdots + X\text{-}P \tag{68}$$

One important aspect of this reaction should be emphasized. In general, it will proceed by a pathway different from the one characterized by Eq. 67. This is true because of the striking specificity of most enzymes. An enzyme which operates on *L* in general will not operate on *L-P*.

Another important aspect of this reaction emerges if we examine the nature of *X-P*. In many biological reactions *X-P* is identified with adenosine triphosphate, abbreviated ATP (Structural formula I).



(I)

We shall for the moment write this in the shortened form A—p~p~p. The distinction between the bonds as indicated above arises from the following considerations. Hydrolysis of the terminal phosphate radical,

$$\text{A—p} \sim \text{p} \sim \text{p} + \text{H}_2\text{O} \rightarrow \text{A—p} \sim \text{p} + \text{H}_3\text{PO}_4$$

is accompanied by a negative free energy change of $\Delta F = -7.8$ kcal. Likewise,

$$\text{A—p} \sim \text{p} + \text{H}_2\text{O} \rightarrow \text{A—p} + \text{H}_3\text{PO}_4$$

with

$$\Delta F = -7.8 \text{ kcal.}$$

However, if the last radical is hydrolyzed the free energy change is some 4 times smaller:

$$\text{A—p} + H_2O \rightarrow \text{A} + H_3PO_4$$

where now

$$\Delta F = -2 \text{ kcal.}$$

This magnitude of free energy change is that associated with a common ester linkage. The obvious conclusion is that when there are three phosphate radicals, the two terminal radicals become modified in such a way that hydrolysis of them is characterized by a large negative ΔF. Thus we write

$$\text{A—p} \sim \text{p} \sim \text{p}$$

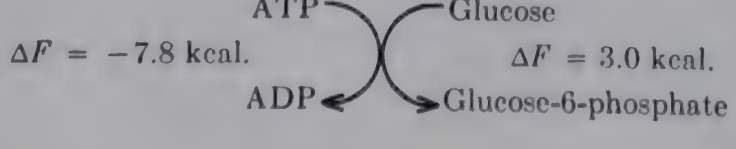
and call the $\sim$ bond a "high-energy bond."

ATP is not the only example of so-called high-energy phosphates. For convenience these compounds may be classified into four principle groups:

(*a*) Carboxyl phosphates (1,3-diphosphoglyceric acid, acetyl phosphate, etc.)
(*b*) Enol phosphates (phosphopyruvic acid, phenylphosphates, etc.)
(*c*) Pyrophosphates (ATP, ADP, etc.)
(*d*) Amino phosphates (creatine phosphate, arginine phosphate, etc.)

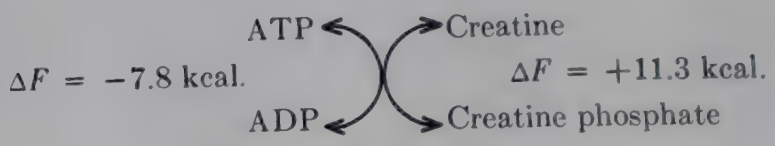
These types of high-energy phosphates are all characterized by a large change in free energy upon hydrolysis, e.g., from —5 to —10 kcal. per mole as compared to the considerably smaller —1 to —3 kcal. per mole for the ordinary ester linkage. Oesper (*9*) discusses in detail why these four main types are characterized by high energy. Essentially types (*a*) and (*c*) owe their high energy to what he refers to as "opposing resonance." Group (*b*) owes its energy to the fact that the enol form is unstable relative to the carbonyl form of the molecule after hydrolysis has taken place. Type (*d*) owes its energy chiefly to the neutralization of the base formed by the hydrolysis.

An example of the type of reaction indicated by Eq. 68 is the hexokinase reaction:



Since ΔF for the phosphorylation is positive, direct introduction of the phosphate group is energetically impossible; however, in the presence of ATP and the enzyme hexokinase the reaction takes place. In the over-all reaction some 4.8 kcal. of free energy is liberated, rendering the reaction quite inefficient energetically speaking. The importance of the reaction is that before glucose can either be stored or metabolized it must first be phosphorylated. The hexokinase reaction is the principle pathway by which this is accomplished.

Another important reaction in which ATP takes part is the Lohmann reaction



where the ΔF's are those for the forward reaction. Since the difference between the two ΔF's is only 3.5 kcal. per mole, this reaction is reversible as indicated by the double arrows. This freely reversible reaction is catalyzed by creatine phosphokinase, an enzyme present in many vertebrate muscles. The importance of this reaction is that it provides a mechanism by which ATP can be taken "out of play." It is a known fact that ATP as such occurs in very small amounts in most tissues. It is therefore significant that larger amounts of the guanidine bases are present. This would suggest that the so-called phosphagens function as local energy pools. Upon demand, these pools yield their energy via the Lohmann reaction producing ATP. It appears that the main stream of biological oxidations is directed towards the production of ATP which, if not immediately used, reacts with free guanidine bases to form phosphagens. When the energy demand of the cell exceeds the rate at which ATP is being generated by the metabolic machinery, local pools provide energy via the Lohmann reaction. After the demand ceases, these pools refill by converting ATP, produced by the metabolic machinery, into ADP and phosphagens.

One more point needs to be emphasized in order to complete this discussion. So far, we have seen how ATP can transfer its terminal group along with some of its intrinsic free energy to other molecules, which then permits reactions to take place that are otherwise not possible. ATP in the presence of an ATP-ase may also undergo a simple splitting or hydrolysis producing free inorganic phosphate. This reaction is accompanied by a loss of free energy equivalent to some 8 kcal. per mole. In the presence of suitable transformers, this free energy may drive endergonic processes essential to life such as osmotic work, electrical work, or mechanical work.

The ATP system is involved in numerous syntheses and metabolic transformations. It is believed to be involved in transmethylation, sulfur assimilation in autotrophic bacteria, and in photosynthesis. This is evidence of its diverse nature. Its importance to biological energetics can hardly be overestimated.

L. Summary of Classical Thermodynamics

We should like to summarize some of the more important ideas pertinent to biological systems as found in classical thermodynamics.

1. The sum of mass and energy in any kind of change is always a constant.
2. There exists a state function, the entropy, which has the following properties:
   a. Entropy is an extensive property. The total entropy of a system is the sum of the entropy of its parts.
   b. The change in entropy may be expressed as

$$dS = d_eS + d_iS \tag{69}$$

   where d_eS is the flow of entropy arising from interactions with the exterior and d_iS is entropy arising from changes within the system itself. Furthermore,

$$d_iS \geqslant 0 \tag{70}$$

   where the equal sign has reference to a reversible process, and the inequality sign pertains to an irreversible process.
   c. The only general criterion of irreversibility is Eq. 70 above; however, state functions like the Gibbs free energy, for example, exist under certain conditions. ΔF is a very useful concept. It represents the maximum amount of work obtainable from any particular process; it determines the direction of reactions; and it is directly related to the equilibrium constant and to the standard oxidation potential. Although the evaluation of ΔF is often based on equilibrium theory, its significance remains the same, whether or not a system is in equilibrium. Furthermore, general expressions may be developed which can be used to evaluate ΔF providing the activities of the products and reactants can be determined. Many times activities cannot be ascertained, and it becomes necessary to make simplifying assumptions; however, the theoretical significance of ΔF remains the same. We belabor this point because there is some confusion about what is meant by the statement that classical thermodynamics does not apply to living systems since they are not equilibrium systems. The problem of accumulation,

storage and expenditure of free energy by organisms is independent of whether or not these organisms are in a steady state condition or in equilibrium states. The evaluation of ΔF is often based on the assumption that the system is in equilibrium when in actuality it is not. Such values, then, must be examined carefully.

III. Thermodynamics of Open Systems

A. Introduction

Living systems avoid the death sentence of the Second Law by virtue of the fact that they are open systems. In this section we wish to explore further the properties of open systems, in particular the steady state, with the idea of indicating methods by which thermodynamic analyses of such systems may be made.

B. Entropy Coupling

In order to present this section in terms currently popular in the literature, we will first consider some fundamental ideas that are attributed to de Donder (*10*). He states the Second Law in the form

$$dq' = T\,dS - dq \tag{71}$$

where dq' is called the "uncompensated heat." Dividing through by T gives

$$\frac{dq'}{T} = dS - \frac{dq}{T} \tag{72}$$

Providing that the process takes place within the system with the environment functioning solely as a heat reservoir, the quantity dq'/T is the total amount of entropy created in the irreversible process dS_{irr}.

Assuming constant T and P

$$dq = dE + P\,dV$$

and

$$dS_{\text{irr}} = \frac{dq'}{T} = dS - (dE - P\,dV) = \frac{T\,dS - (dE - P\,dV)}{T} \tag{73}$$

Now

$$dF = dE + P\,dV - T\,dS$$

therefore

$$dS_{\text{irr}} = \frac{-dF}{T} \tag{74}$$

The rate of entropy production is

$$\frac{dS_{\text{irr}}}{dt} = -\frac{1}{T}\frac{dF}{dt} \tag{75}$$

de Donder now makes an important hypothesis: assuming irreversibility is due solely to chemical reaction, the entropy production depends only upon the "degree of advancement" of the reaction. The degree of advancement is defined in the following manner. Suppose we have a single reaction taking place. This may be written

$$\sum_i \nu_i M_i = 0$$

where M_i represents the chemical formulae of the various participating substances and ν_i is their stochiometric coefficients. Let n_i^0 equal the number of moles of i at time $t = 0$, and n_i equal the number of moles of i at time t. The degree of advancement is defined by

$$\xi = \frac{(n_i^0 - n_i)}{\nu_i} \tag{76}$$

and thus

$$d\xi = dn_i/\nu_i \tag{77}$$

Obviously $d\xi/dt$ is the rate of reaction which we shall denote by v. Considering the expression for free energy

$$dF = -S\,dT + V\,dP + \sum_i \mu_i\,dn_i \tag{78}$$

we have at constant T and P

$$dF = \sum_i \mu_i\,dn_i \tag{79}$$

Thus,

$$\frac{dS_{\text{irr}}}{dt} = -\frac{1}{T}\sum_i \mu_i \frac{dn_i}{dt} \tag{80}$$

This is a general equation and holds for any number of reactions obtaining in the system. We may now write Eq. 80 in the form

$$\frac{dS_{\text{irr}}}{dt} = -\frac{1}{T}\cdot v \cdot \sum_i \nu_i\mu_i \tag{81}$$

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The quantity $-\sum_i \nu_i \mu_i$ is called the affinity and is given the symbol A. Letting $\theta = dS_{irr}/dt$ we have

$$\theta = \frac{1}{T} A \cdot v \tag{82}$$

For j simultaneous reactions the equation modifies to

$$\theta = \frac{1}{T} \sum_j A_j v_j \tag{83}$$

The Second Law then states that

$$\theta = \frac{1}{T} \sum_j A_j v_j > 0 \tag{84}$$

Thus we see that we have a bilinear form of the affinities and rates of chemical reactions. Suppose $j = 1, 2$. Then it may happen that $A_1 v_1 < 0$ and $A_2 v_2 > 0$ provided that

$$A_1 v_1 + A_2 v_2 > 0 \tag{85}$$

Both reactions are then said to be coupled. This permits one of the reactions to progress in the direction contrary to that prescribed by its own affinity, or change in ΔF. Thus, as Prigogine (*11*) suggests, in thermodiffusion the diffusion of matter against a concentration gradient is characterized by a negative entropy production, but this effect is compensated by positive entropy production due to flow of heat. We see that coupled reactions are extremely interesting in the case of biological systems, because they are concerned with the apparent decrease in entropy and an evolution always towards the more complex. The following question then arises: is the decrease of entropy compensated by coupled processes within the organism itself, or are other processes involved? We would like a general scheme to provide us with an analysis revealing which processes may be coupled in the organism. Here the theory of Onsager appears to offer a promising approach. But before proceeding to a consideration of his theory, we will first investigate entropy production in natural processes.

C. Entropy Production

1. *Entropy Production in Flow of Electric Current*

For electrical conduction the rate of performance of work is

$$\frac{dW}{dt} = -i\nabla\phi \tag{86}$$

where i is the current density and ϕ is the potential. ∇ is the differential operator

$$\frac{\partial}{\partial x} + \frac{\partial}{\partial y} + \frac{\partial}{\partial z}$$

Assuming the electrical energy to be entirely dissipated as heat to the surroundings at temperature T, then the rate of entropy production is

$$\frac{dS}{dt} = \frac{1}{T}\frac{dW}{dt} \qquad \text{or} \qquad T\frac{dS}{dt} = -i\nabla\phi \tag{87}$$

We may write this as

$$T\theta = J \cdot X \tag{88}$$

where

$$J = i$$
$$X = -\nabla\phi$$

2. *Entropy Production in Isothermal Diffusion*

Imagine a small volume element as represented in Fig. 3. Suppose that there are n_i moles of substance passing through face 1 and an

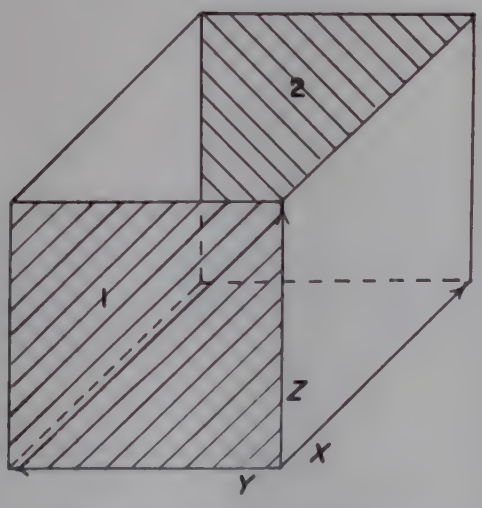


FIG. 3. Volume element.

amount of $[n_i + (\partial n_i/\partial x)\,dx]$ leaving the other opposite face, e.g., face 2. The corresponding free energy increase would be

$$\Delta F = n_i\mu_i + \left[\frac{\partial \mu_i}{\partial x}\,dx - \mu_i\right] n_i = n_i\frac{\partial \mu_i}{\partial x}\,dx \tag{89}$$

Let J_i be the flux of i per unit time per unit area. Then

$$J_i = \frac{1}{A}\frac{dn_i}{dt} \tag{90}$$

where A represents the area. The rate of increase in free energy is thus

$$\frac{dF}{dt} = J_i A \frac{\partial \mu_i}{\partial x} dx \tag{91}$$

For several species this expression is summed over all transfers. Since

$$\frac{dS_{\text{irr}}}{dt} = -\frac{1}{T}\frac{dF}{dt}$$

we have

$$\frac{dS_{\text{irr}}}{dt} = -\frac{1}{T}\sum_i J_i A \frac{\partial \mu_i}{\partial x} dx \tag{92}$$

or per unit volume

$$T\theta = -\sum_i J_i \cdot \frac{\partial \mu_i}{\partial x} \tag{93}$$

In general, for three dimensions,

$$T\theta = -\sum_i J_i \cdot \nabla \mu_i \tag{94}$$

Again we see that this may be written

$$T\theta = J \cdot X$$

where

$$J = J_i$$
$$X = -\nabla \mu_i$$

The general method is clear by which we may calculate the entropy production of other processes. For the flow of energy the result is also expressible in the form

$$T\theta = J \cdot X$$

where

$J =$ the energy flow per unit area per unit time and

$$X = T\nabla \cdot 1/T = -\nabla T/T$$

3. *Entropy Production in Open Systems*

Consider the model of a cell presented in Section II,C. We now wish to consider the production of entropy in such an open system. To do this we use the Gibbs formula in the form

$$dS = \frac{dE}{T} + \frac{P}{T} dV + \sum_i \frac{\mu_i}{T} dn_i \tag{95}$$

where n_i is the number of moles of the ith component. In an open system we may consider dn_i, the change in the number of moles of the ith component, to be due to two sources: the part $d_e n_i$, supplied from the exterior and $d_i n_i$, the part due to changes inside the system. We must also look at the expression which represents conservation of energy in an open system. It is clear that the First Law as stated for closed systems must be modified to include exchange of matter with the exterior. Thus,

$$dE = d\Phi - P\,dV \tag{96}$$

The function $d\Phi$ represents a resultant flow of energy per unit time arising from exchange or transfer of heat and matter. The notation is that used by Prigogine (*11*). Recall that

$$dn_i = \nu_i\,d\xi = d_e n_i + d_i n_i \tag{97}$$

Gibb's formula then becomes

$$dS = \frac{d\Phi}{T} - \frac{P\,dV}{T} + \frac{P\,dV}{T} - \sum_i \frac{\mu_i}{T}(d_e n_i + d_i n_i)$$

But

$$dn_i = d_e n_i + \sum_i \nu_i\,d\xi$$

Thus

$$\begin{aligned} dS &= \frac{d\Phi}{T} - \sum_i \frac{\mu_i \nu_i}{T}\,d_i n_i - \sum \frac{\mu_i}{T}\,d_e n_i \\ &= \frac{d\Phi}{T} - \sum_i \frac{\mu_i}{T}\,d_e n_i + \frac{A\,d\xi}{T} \end{aligned} \tag{98}$$

Let us apply the above ideas to our system which consists of a cell representing the α-phase, and the ground substance representing the β-phase. This system is enclosed in the organism. We shall consider it to be a closed system. Summing Eq. 98 for both phases

$$dS = \frac{d^\alpha\Phi}{T^\alpha} + \frac{d^\beta\Phi}{T^\beta} - \sum_i \left(\frac{\mu_i{}^\alpha}{T^\alpha} - \frac{\mu_i{}^\beta}{T^\beta}\right) d_e n_i + \frac{A^\alpha\,d\xi}{T^\alpha} + \frac{A^\beta\,d\xi}{T^\beta} \tag{99}$$

where A^α and A^β are the affinities of reactions taking place in both phases. $d^\alpha\Phi$ represents heat coming from the exterior, $d_e Q$, plus the energy flow, $d_i{}^\alpha\Phi$, from the β-phase. Thus,

$$d^\alpha\Phi = d_e{}^\alpha Q + d_i{}^\alpha\Phi \tag{100}$$

Likewise

$$d^{\beta}\Phi = d_e{}^{\beta}Q + d_i{}^{\beta}\Phi \tag{101}$$

Thus,

$$dS = \frac{d_e{}^{\alpha}Q}{T^{\alpha}} + \frac{d_e{}^{\beta}Q}{T^{\beta}} + \left(\frac{1}{T^{\alpha}} - \frac{1}{T^{\beta}}\right) d_i{}^{\alpha}\Phi \sum_i \left(\frac{\mu_i{}^{\alpha}}{T^{\alpha}} - \frac{\mu_i{}^{\beta}}{T^{\beta}}\right) d_e n_i{}^{\alpha} + \frac{A^{\alpha}\, d\xi^{\alpha}}{T^{\alpha}} + \frac{A^{\beta}\, d\xi^{\beta}}{T^{\beta}} \tag{102}$$

This may be written

$$dS = d_e S + d_i S \tag{103}$$

where

$$d_e S = \frac{d_e{}^{\alpha}Q}{T^{\alpha}} + \frac{d_e{}^{\beta}Q}{T^{\beta}} \tag{104}$$

represents the entropy flow, and

$$d_i S = \left(\frac{1}{T^{\alpha}} - \frac{1}{T^{\beta}}\right) d_i{}^{\alpha}\Phi - \sum_i \left(\frac{\mu_i{}^{\alpha}}{T^{\alpha}} - \frac{\mu_i{}^{\beta}}{T^{\beta}}\right) d_e n_i + \frac{A^{\alpha}\, d\xi^{\alpha}}{T^{\alpha}} + \frac{A^{\beta}\, d\xi^{\beta}}{T^{\beta}} \tag{105}$$

represents the entropy production arising from a transport of heat and matter between the two phases, plus that from chemical reactions in the two phases. By the Second Law, $d_i S \geqslant 0$. The rate of entropy production is therefore

$$\frac{d_i S}{dt} = \left(\frac{1}{T^{\alpha}} - \frac{1}{T^{\beta}}\right) \frac{d_i{}^{\alpha}\Phi}{dt} - \sum_i \left(\frac{\mu_i{}^{\alpha}}{T^{\alpha}} - \frac{\mu_i{}^{\beta}}{T^{\beta}}\right) \frac{d_e n_i{}^{\alpha}}{dt} + \frac{A^{\alpha} v^{\alpha}}{T^{\alpha}} + \frac{A^{\beta} v^{\beta}}{T^{\beta}} \geqslant 0 \tag{106}$$

Comparing this to Eq. 84 we see that the form is the same, e.g., bilinear in the rates and affinities.

D. Onsager's Theory (*12*)

We have made considerable effort to show that for many processes the entropy production may be written in the form

$$T\theta = \sum_i J_i \cdot X_i \tag{107}$$

We should now like to take a closer look at the J's by returning to our model in Section II,C. Let us consider a reaction taking place within the α-phase which produces a considerable amount of heat which must

be dissipated to the β-phase. At the same time, suppose there is also present a diffusable substance in the α-phase which passes to the β-phase. Let J_1 represent the rate of energy flow and J_2 represent the rate of matter flow. The rate of each is regarded as being proportional to a "thermodynamic force." In the case of energy, we have already seen that this force is $-\nabla T$. In the case of diffusion it was seen to be $-\nabla \mu_i$. When two or more such processes occur simultaneously, the rates of the separate processes are considered to be proportional not only to the corresponding force, but proportional also to other forces. Thus, in the above case we write

$$\begin{aligned} J_1 &= L_{11}X_1 + L_{12}X_2 \\ J_2 &= L_{21}X_1 + L_{22}X_2 \end{aligned} \tag{108}$$

The coefficients L_{11} and L_{22} are in the form of a thermal conductance and of a diffusion coefficient, respectively. L_{12} and L_{21} are "coupling" coefficients which may or may not be equal to zero.

In general

$$J_i = \sum_k L_{ik}X_k \tag{109}$$

Such equations are applicable to all systems in which there is a coupling, providing that the J's are linear functions of the X's. Such equations have become known as the "phenomenological equations," a term used by Onsager, or as the "thermodynamic equations of motion," a term proposed by Eckart (*13*).

Returning to our simple model, substituting Eq. 108 into Eq. 107 gives

$$\begin{aligned} T\theta &= (L_{11}X_1 + L_{12}X_2)X_1 + (L_{21}X_1 + L_{22}X_2)X_2 \\ &= L_{11}X_1^2 + (L_{12} + L_{21})X_1X_2 + L_{22}X_2^2 > 0 \end{aligned} \tag{110}$$

The Second Law imposes the restrictions that

$$L_{11} > 0, \qquad L_{22} > 0$$

and

$$(L_{12} + L_{21})^2 < 4L_{11}L_{22} \tag{111}$$

Providing Eq. 111 holds, L_{12} and L_{21} may be negative; L_{11} and L_{22} must always be positive.

Onsager made a significant contribution to the theory when he showed that

$$L_{ik} = L_{ki} \tag{112}$$

if the following conditions are satisfied: (*a*) the thermodynamic forces are chosen in such a way that Eq. 107 holds; and (*b*) these forces also satisfy Eq. 109.

E. Applications of the Theory

Onsager's reciprocal relations permit a closer look at some of the coupling between irreversible processes. As an example let us extend an analysis developed by Prigogine (*11*) to our simple model of a cell. Suppose the α- and β-phases to have uniform concentrations throughout and to differ only with respect to pressure and electrical potentials. It is a well-known phenomenon that in many cells, a potential difference exists across the membrane. In nerve cells, for example, in the resting state a transmembrane potential exists of the order of 100 mv.

The entropy production arising from the transfer of material from the α-phase to the β-phase is given by

$$d_iS = \frac{1}{T}\sum_k \bar{A}_k\, d\xi = -\frac{1}{T}\sum_k \bar{A}_k\, dn^\alpha \tag{113}$$

where $\bar{A}$ is the electrochemical affinity defined by

$$\bar{A} = (\mu_k{}^\alpha - \mu_k{}^\beta) + Z_kF(\epsilon^\alpha - \epsilon^\beta) \tag{114}$$

The subscript k refers to the kth component. In this equation $(\epsilon^\alpha - \epsilon^\beta)$ represents the transmembrane potential $\Delta\epsilon$. Since

$$\left(\frac{\partial \mu_k}{\partial P}\right)_{T,n_k} = V_k$$

or for a finite change,

$$\Delta\mu_k = V_k\Delta P \tag{115}$$

where V_k is the partial molar volume of constituent k, then

$$\frac{d_iS}{dt} = -\frac{1}{T}\sum_k V_k\Delta P\,\frac{dn^\alpha}{dt} - \frac{1}{T}\sum_k Z_kF\,\frac{dn^\alpha}{dt}\,\Delta\epsilon \tag{116}$$

Defining

$$J = -\sum_k V_k\,\frac{dn^\alpha}{dt} = \text{the resultant flow of matter}$$

and

$$I = -\sum_k Z_k\Delta\epsilon\cdot\frac{dn}{dt} = \text{the current due to the flow of ions.}$$

it follows that

$$\frac{d_i S}{dt} = \frac{J\Delta P}{T} + \frac{I\Delta\epsilon}{T} \tag{117}$$

Then the thermodynamic equations of motion are given by

$$I = L_{11}\frac{\Delta\epsilon}{T} + L_{12}\frac{\Delta P}{T} \tag{118}$$

$$J = L_{21}\frac{\Delta\epsilon}{T} + L_{22}\frac{\Delta P}{T} \tag{119}$$

When $I = 0$

$$\frac{\Delta\epsilon}{\Delta P} = -\frac{L_{12}}{L_{11}} \tag{120}$$

This effect is known as the streaming potential and is defined as the potential difference per unit pressure difference when there is no electrical current.

When $\Delta P = 0$

$$\frac{J}{I} = \frac{L_{21}}{L_{11}} \tag{121}$$

This is called the electroosmotic effect and is defined as the flow of matter per unit electrical current when the pressure is uniform.

When $J = 0$

$$\frac{\Delta P}{\Delta\epsilon} = -\frac{L_{21}}{L_{22}} \tag{122}$$

This is called the electroosmotic pressure and is defined as the pressure difference per unit potential difference when there is no flow of matter.

When $\Delta\epsilon = 0$

$$\frac{I}{J} = \frac{L_{12}}{L_{22}} \tag{123}$$

This is called the streaming effect. It is defined as electrical current per unit flow of matter when the voltage is zero.

These several effects may be studied independently by various experimental methods. Onsager's reciprocal relations lead to the following two results

$$\left(\frac{\Delta\epsilon}{\Delta P}\right)_{I=0} = -\left(\frac{J}{I}\right)_{\Delta P=0} \tag{124}$$

and

$$\left(\frac{\Delta P}{\Delta\epsilon}\right)_{J=0} = -\left(\frac{I}{J}\right)_{\Delta\epsilon=0} \tag{125}$$

The first is recognized as Saxen's relationship which was first derived with kinetic theory using a much more restrictive model. Unfortunately, such methods do not yield explicit values for the coefficients but the importance of the method lies in its ability to relate effects which may appear to be independent.

This completes, for the time being, the brief excursion into some of the ideas of "classical" irreversible thermodynamics. Much could be said about the validity of the underlying assumptions (*14–16*), especially concerning the reciprocal relations. In general, the system under consideration must be near equilibrium. Exactly how near the equilibrium state the system must be, however, in order that the postulated linearity will suffice as an adequate approximation is not always made clear. In diffusion and chemical reactions, for example, the approximations described above are almost never satisfactory. In these cases, any attempt to improve the theory by relating the forces and fluxes by a nonlinear equation renders the whole approach impractically cumbersome.

Recently, irreversible processes have been treated by absolute rate theory (*17*). The results derived are found to continue to hold for large departures from equilibrium in a range where the Onsager reciprocal relations are no longer applicable. After the section on rate theory has been presented, we shall indicate the essential features of such a treatment.

IV. Reaction Rates in Chemical and Biological Systems

Protoplasm in order to function properly must maintain a constant chemical composition within rather narrow limits, which implies the existence of a precise control over the rates of the many reactions involved in metabolism. If, for example, the rates of reactions supplying energy to the organism decrease, the general metabolism and activity will slow down. On the other hand, if energy supplying reactions take place too fast, food will be oxidized faster than the organism can replenish its supply. The study of reaction rates is much more complicated than the study of thermodynamics since, in addition to a knowledge of the initial and final states, we must also know the details of intermediate products and reaction mechanisms.

A. Effect of Temperature on Reaction Rate

As a result of experience we know that, in general, rates of chemical reactions increase as the temperature is raised. Biological reactions, of course, behave in much the same way. The range of temperature over which biological systems can exist in a metabolically active form is ex-

tremely limited. Ordinary plants and animals carry out normal life activities only over the approximate range of 0–50°. Some organisms are, of course, less sensitive. Some thermophilic algae and bacteria live in hot springs at temperatures up to 70° or perhaps even higher. Mammals, on the other hand, are very sensitive to body temperatures only a few degrees higher than normal. In the other direction, almost all organisms are inactive as temperatures of 0° are approached, although some plants continue to grow slowly even at several degrees below this.

Biologists often express the effect of temperature on the rate of a reaction in terms of the *temperature coefficient* or Q_{10}. This is the ratio between the rate of a reaction at one temperature and its rate at a temperature 10° lower as given by the equation

$$Q_{10} = \frac{k_{t+10}}{k} \tag{126}$$

where k is the rate constant and t is temperature. A more useful expression is given by the van't Hoff equation

$$Q_{10} = \left(\frac{k_2}{k_1}\right)^{10/(t_2 - t_1)}$$

or

$$\log Q_{10} = \frac{10}{t_2 - t_1} \log \frac{k_2}{k_1}$$

where k_1 and k_2 are the reaction rates at temperatures t_1 and t_2, respectively. The Q_{10} for most biological processes is approximately 2. The value of Q_{10} will vary somewhat with temperature, therefore the temperature over which Q_{10} has been measured should always be given.

Temperature, then, is a very important factor in determining the rate of a reaction. The question immediately arises as to the mechanism involved. As temperature increases, the kinetic energy of molecules is increased. However, the small increase in the average molecular kinetic energy cannot account for the observed increase in reaction rate. Arrhenius proposed that the rate of a reaction is controlled, not by the average kinetic energy of the reacting molecules, but by the concentration of molecules possessing more than a critical amount of energy (the so-called *energy of activation*). The effect of temperature on the increase in concentration of molecules with the proper energy of activation is similar to the effect of temperature on the rate of reaction. Arrhenius found a linear relation between the rate constant for a reaction and the reciprocal of the absolute temperature. This led to the

formulation of the so-called Arrhenius equation which is usually written in the form

$$k = Ae^{-E/RT} \tag{127}$$

where k is the specific rate constant, E is the heat constant between activated and inert molecules (usually termed the *energy of activation*), R is the gas constant, T the absolute temperature, and A is the so-called *frequency factor*. The Arrhenius equation is generally accepted as the relationship representing the temperature dependence of specific reaction rates of most chemical reactions and of many biological processes. Provided the temperature range is not large, E and A may be taken as constant. The energy of activation, E, represents the energy that the molecule in the initial state of the process must acquire before it can take part in the reaction. In its simplified form, therefore, the problem of making absolute calculations of reaction rates involves two independent aspects: the determination of the energy of activation and of the frequency factor. The rate constant is also a function of pressure. This is not taken into account in the Arrhenius equation but is in absolute rate theory.

B. Absolute Reaction Rate Theory

The method of calculating the frequency factor A using the "theory of absolute reaction rates" (*18*) is based on the fundamental concept that reactants combine to form a critical intermediate, called the activated complex, to which equilibrium theory may be applied. If one plots on independent coordinates all of the distances which must be specified to fix the potential energy of the activated complex, and in an independent direction plots the corresponding energy, then a potential surface in configuration space is obtained which is similar to an ordinary landscape. On such a surface basins correspond to compounds, and saddle points in the passes between the low regions correspond to activated complexes. If, from an initial low region, one climbs to the pass and then descends by the "water course" into the adjoining low region, this path corresponds to the reaction coordinate. A plot of energy against distance along the reaction coordinate for any reaction has the general appearance as shown in Fig. 4.

The activated complex is to be regarded as an ordinary molecule, possessing all the usual thermodynamic properties, with the exception that motion in one direction, i.e., along the reaction coordinate, leads to decomposition at a definite rate. With these assumptions, it is possible to derive the concentration of and the rate at which the activated complexes pass through the critical activated configuration by statistical

methods. The product of these quantities is equal to the reaction rate.

Consider a process involving the reactants A, B, etc., which form the activated complex $M^{\ddagger}$ in the reaction:

$$A + B + \cdots \rightarrow M^{\ddagger} + Q + R \rightarrow \text{Products} \tag{128}$$

The superscript ‡ will be used to designate the activated complex or its thermodynamic parameters. For many reactions the species Q and R do not occur, so that for brevity we will omit them hereafter. The rate of the reaction is equal to the concentration of activated complexes at the top of the barrier multiplied by the frequency of crossing the barrier.

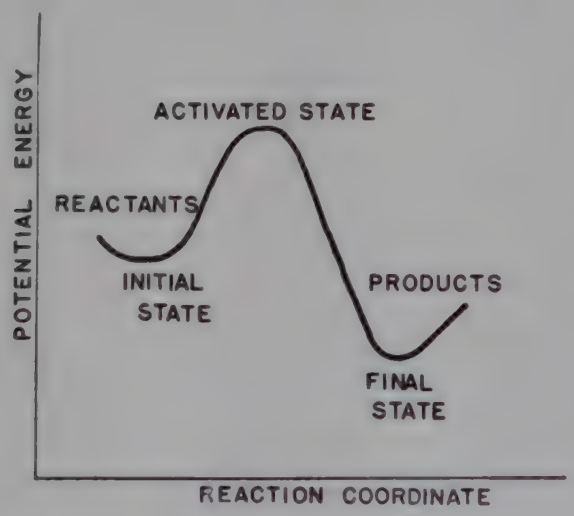


FIG. 4. Potential energy diagram.

If c^* is the number of activated complexes in unit volume lying in a length δ along the reaction coordinate at the top of the barrier, and $\bar{v}$ is the mean velocity of crossing, then $\bar{v}/\delta$ is the frequency with which activated complexes pass over the barrier. Since one half of the equilibrium number of complexes will be moving in the forward direction, we have for the reaction rate:

$$\text{Rate of reaction} = \tfrac{1}{2} c^* \frac{\bar{v}}{\delta} \kappa \tag{129}$$

The transmission coefficient, κ, is a factor which is required since not every crossing need end in a successful reaction. The activated complexes differ from normal molecules only in that one of the degrees of vibrational freedom is replaced by translational motion along the reaction coordinate. These complexes can, however, be thought of as normal molecules by writing their concentration at the top of the barrier as:

$$c^* = \frac{c^{\ddagger}(2\pi m^{\ddagger} kT)^{1/2}\delta}{h} \tag{130}$$

where $m^{\ddagger}$ is the effective mass of the activated complex in the coordinate of reaction. The factor $(2\pi m^{\ddagger} kT)^{1/2}/h$ is the partition function for

the reaction coordinate. Thus while c^* is the concentration of activated complexes per length δ along the reaction path, $c^\ddagger$ is this same concentration when δ has the particular value

$$\delta = \frac{h}{(2\pi m^\ddagger kT)^{1/2}} \tag{131}$$

By the use of classical methods the mean velocity ($\bar{v}$) of crossing the barrier in one direction, i.e., in the direction of decomposition, is found to be

$$\bar{v} = \frac{\int_0^\infty (nh/2m^\ddagger\delta) \cdot e^{-n^2h^2/8\delta^2 m^\ddagger kT}}{\int_0^\infty e^{-n^2h^2/8\delta^2 m^\ddagger kT}} = \sqrt{\frac{2kT}{\pi m^\ddagger}} \tag{132}$$

Equation 129 becomes

$$\text{Rate of reaction} = \tfrac{1}{2}c^\ddagger(2\pi m^\ddagger kT)^{1/2} \cdot \delta \left(\frac{2kT}{\pi m}\right)^{1/2} \frac{1}{\delta}\kappa = \kappa c^\ddagger \frac{kT}{h} \tag{134}$$

The striking consequence of the foregoing treatment is that the effective rate of crossing the energy barrier by the activated complexes is equal to kT/h, which is a universal frequency, dependent only on temperature and independent on the nature of the reactants or of the type of reaction.

Assuming that there is a virtual equilibrium between activated complexes and reactants as for any equilibrium, we have

$$\frac{a^\ddagger}{a_A a_B} = K_a{}^\ddagger$$

Hence,

$$c^\ddagger = \frac{c_A c_B \gamma_A \gamma_B}{\gamma^\ddagger} \cdot K_a{}^\ddagger = c_A c_B K^\ddagger \tag{135}$$

where γ_A, γ_B, and $\gamma^\ddagger$ are the activity coefficients of the respective species. For convenience, we have absorbed the activity coefficients into an equilibrium constant $K^\ddagger$. If k' is the specific reaction rate, the velocity of the reaction under consideration is given in the familiar manner by

$$\text{Rate of reaction} = k' c_A c_B \ldots \tag{136}$$

Thus from Eqs. 134 and 136

$$\kappa c^\ddagger \frac{kT}{h} = k' c_A c_B \ldots$$

Or

$$k' = \kappa \frac{kT}{h} \cdot \frac{c^{\ddagger}}{c_A c_B} \cdot \cdot \cdot = \kappa \frac{kT}{h} K^{\ddagger} \tag{137}$$

where $K^{\ddagger}$ is the constant for the equilibrium,

$$A + B \rightleftharpoons M^{\ddagger}$$

existing between the reactants and the activated complex. The constant $K^{\ddagger}$ is exactly analogous to any other equilibrium constant. By appropriate concern for activity coefficients it may be related to $\Delta F^{\ddagger}$, $\Delta H^{\ddagger}$, and $\Delta S^{\ddagger}$, the standard free energy, heat constant, and entropy changes, respectively, accompanying the formation of the activated state from the reactants, by means of the familiar thermodynamic relationships. In this manner, Eq. 137 can be written

$$k' = \kappa \frac{kT}{h} \cdot e^{-\Delta F^{\ddagger}/RT} \tag{138}$$

where

$$K = e^{-\Delta F^{\ddagger}/RT} = e^{-\Delta H^{\ddagger}/RT} \cdot e^{\Delta S^{\ddagger}/R}$$

Thus:

$$k' = \kappa \frac{kT}{h} \cdot e^{-\Delta H^{\ddagger}/RT} \cdot e^{\Delta S^{\ddagger}/R} \tag{139}$$

The constant, $K^{\ddagger}$, is the ratio of the partition functions of the activated complex to that for the reactants. The partition functions of any atom or molecule, including the activated complex, have the value

$$f = \sum_i e^{-E_i/kT}$$

where E_i is the energy of the state of the molecule and the summation is over all states. As the number of molecules of a species is always proportional to its partition function, we have an invaluable means of expressing equilibrium constants as ratios of the appropriate partition functions. Since the energy of a molecule can be written as the sum of energies for its degrees of freedom and this energy occurs in the exponent of the partition function, it follows that the over-all partition function can be written as a product of partition functions for all the degrees of freedom.

The partition function for three translational degrees of freedom is

$$f_{3t} = \frac{[(2\pi mkT)^{3/2}]V}{h^3} \tag{140}$$

in which V is the volume of the container and the other symbols a
the same as we have used before in Eq. 130. Expressions for the pa
tion functions are derived from quantum and statistical-mechanic
theory. Since the equilibrium constant for any system can be expres
in terms of the partition functions of the molecules concerned, Eq. 1
can be written

$$k' = \kappa \left(\frac{kT}{h}\right) \frac{F^{\ddagger}}{F_A F_B \cdots} \cdot e^{-E_0/RT} \qquad (14$$

Here $F^{\ddagger}$ is the partition function per unit volume of the activated co
plex; F_A and F_B are the partition functions of the reactants, and E_0
the difference in zero-level energy, e.g., the activation energy at a
solute zero which has merely been taken out of the partition functio
Physical data necessary for computing partition functions may be o
tained from spectroscopy, and from a knowledge of the mass and stru
ture of the reacting molecules.

From Eq. 138 and 139 it is apparent that the term $(F^{\ddagger}/F_A F_B \cdots$
in Eq. 141 involves principally the entropy of activation, $\Delta S^{\ddagger}$. Fr
quently, especially for unimolecular reactions, the activated complex
a looser structure than the reactants and the entropy of activation
positive. In some reactions, however, the activated complex is a mo
rigid structure than the reactants, and the entropy of activation is neg
tive. The specific rate in all cases, when the transmission coefficient κ
unity, depends upon the product of the exponential terms involvi
entropy and heats of activation, or in other words, upon the free ener
change in passing from the normal state to the activated complex, t
rate of decomposition of the activated complex always being equal
kT/h.

C. Application of Absolute Reaction Rate Theory to Biological Processes

1. *Importance of the Activation Energy*

The cardinal role played by the free energy of activation in ra
processes, as clearly revealed by absolute rate theory, affords a fund
mental way of viewing many reactions. Consider, for example, t
hydrolysis reaction of ATP presented in an earlier section. In terms
rate theory, the reaction may be written

$$\text{ATP} + \text{H}_2\text{O} \rightarrow \left[\text{Enz} \cdots \text{ATP} \cdots \text{O}\begin{matrix}\text{H}\\ \\ \text{H}\end{matrix}\right]^{\ddagger} \rightarrow \text{ADP} + \text{P}_i$$

It is assumed that the enzyme combines with the substrates to form an activated complex which is in equilibrium with the reactants. The rate at which the activated complexes cross the potential barrier in the forward direction to yield the products represents the rate of reaction. The process may be represented by Fig. 5.

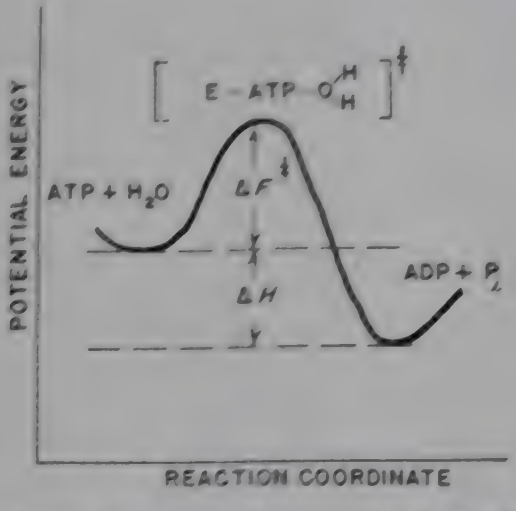


FIG. 5. Potential energy diagram for hydrolysis of ATP.

The first important point to understand is that $\Delta F^{\ddagger}$ is a measure of the ease with which the reaction proceeds. It is a direct measure of the kinetic, as distinguished from the thermodynamic, escaping tendency of the phosphate group. It represents the amount of energy the reactants must acquire before they are able to react. If $\Delta F^{\ddagger}$ is large there will be little tendency to react, while if $\Delta F^{\ddagger}$ is small the tendency to react will be great.

The other important point is that once the activation energy is supplied, the reaction will proceed with a concomitant change in enthalpy, ΔH. If the entropy change of the reaction can be determined, the free energy change for the hydrolysis may be calculated from the expression

$$\Delta F = \Delta H - T\Delta S$$

Recent determinations of ΔF give a value of -7.8 kcal. for this reaction. "High-energy" compounds are characterized by hydrolytic free-energy changes of this order of magnitude. The standard free energy of hydrolysis with the sign reversed is sometimes spoken of as the "bond energy." This is in contradistinction to the usual concept of bond energy of physical chemistry. The "bond energy" concept has led to the treatment of ΔF as a tangible object, a bundle of energy, which may be taken from one molecule and attached to another. This concept is quite erroneous. In the case of a group transfer, the free energy for the over-all reaction is more accurately considered as the sum of two

reactions, one a hydrolysis, the other a dehydration. Thus, in the case of the hexokinase reaction,

$ATP + H_2O \rightarrow ADP + H_3PO_4$	$\Delta F = -7.8$ kcal.
$Glucose + P_i \rightarrow Glucose\text{-}6\text{-}phosphate + H_2O$	$\Delta F = 3.0$ kcal.
$ATP + Glucose \rightarrow Glucose\text{-}6\text{-}phosphate + ADP$	$\Delta F = -4.8$ kcal.

Calculating the equilibrium constant gives a value of

$$K = 2.5 \times 10^3$$

This value assures that the phosphorylation of glucose proceeds essentially to completion.

The possible synthesis of ATP from ADP and inorganic phosphate (P_i) has been sometimes considered as a function of the free energy of an oxidation reaction. Thus, if the standard free energy of an oxidation is of the order of magnitude of 10 kcal. per mole, it is concluded that one ATP molecule may be generated in a coupled reaction. If the free energy of the oxidation is several times as large, then the total free energy divided by the "bond energy" gives the maximum number of ATP molecules that may be formed. Biochemical arithmetic of this type is a completely invalid application of the free energy concept. Analysis using rate theory shows that it is not the free energy of a reaction that determines whether or not a reaction will proceed, but rather the free energy of activation.

It is important to note that the term "bond energy" in so-called high-energy phosphate bonds was created to provide a convenient means for expressing the free energy changes of hydrolysis that can be coupled biologically. This coupling is usually effected by enzymes by lowering the free energy of activation. Absolute rate theory thus provides a deeper insight into the mechanisms of energy coupling.

2. *The Effect of External Forces on Reactions*

We are now in a favorable position to discuss one of the central problems in biology: how does the cell microstructure modify results derived for ideal systems?

In general, cell microstructure will manifest itself by exerting an external force that will act on some cellular process in such a way that it will either tend to aid or hinder the process by increasing or decreasing the free energy of the initial or final configuration with respect to the activated state. In other words, it alters the free energy of activation. This may be represented by the simple diagram in Fig. 6.

For simplicity of treatment consider a two-component system governed by a single unimolecular rate constant having a symmetrical bar-

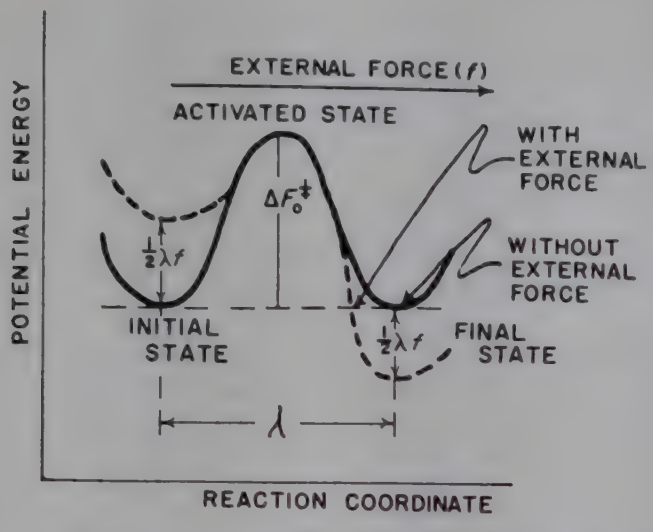


FIG. 6. Effect of external forces on reaction rates.

rier. The basic premise is that an external force acting on a single unit process provides an additional amount of work, i.e.,

$$W = \tfrac{1}{2}\lambda f$$

which hinders or aids the process. The net linear velocity with which molecules jump over the barrier is

$$v = (k_f - k_b) = k_0\lambda(e^{W/kT} - e^{-W/kT})$$

where k_0 is the specific rate constant without the applied force. This may also be written in the form

$$v = \lambda k_0 2 \sinh (W/kT)$$

In many cases, the approximation

$$W \ll kT$$

is a good one. This allows the hyperbolic sine function to be expanded in a power series. Only the first term is retained. Thus,

$$v = \lambda k_0\left(\frac{2W}{kT}\right)$$

With the above definition of work, this equation is readily generalized to take into account the various forces acting on the process:

$$v = \left(k_0\lambda \sum_i f_i\right) \Big/ kT$$

The essential point is that a difference in free energy between an equilibrium position and the activated state makes a corresponding change in the free energy of activation. A great number of processes involve the passage of a point in configuration space over a series of suc-

cessive free energy barriers. Examples are membrane permeability, diffusion-controlled processes, chain reactions, and nucleation. It is of interest, therefore, that Giddings and Eyring (*19*) have derived a general solution for the steady state rate of such multi-barrier processes. Interesting results have been obtained treating diffusion (*20*), membrane potentials (*21*), and heart action (*22*) using the method of reaction rates.

The treatment also lends itself very nicely to the development of irreversible thermodynamics. A temperature gradient modifies the absolute reaction rate equation by the appropriate substitution of temperature into the partition function for the normal and activated states. When the free energy change is small the reaction rate treatment reduces to the standard irreversible thermodynamic formulation. The absolute rate expression, however, continues to hold for large departures from equilibrium in a range where the Onsager reciprocal relations are no longer applicable.

3. *Bioluminescence* (*22*)

As a final application of absolute reaction rate theory to biological phenomena we will consider the rate of bacterial luminescence.

The theoretical expression is readily derived if it is assumed that the observed rate of an enzyme reaction at different temperatures is governed primarily by (*a*) the activation energy of the catalytic reaction, and (*b*) an equilibrium between native and denatured forms of the catalyst. For the present discussion a third important process, the irreversible denaturation of the catalyst, will be omitted.

Let A_n represent the native, active form of luciferase in equilibrium with A_d, the reversible denatured inactive form, and let A_0 equal the total amount, $A_n + A_d$, under conditions where essentially none of the enzyme undergoes irreversible destruction. We have

$$A_n \rightleftharpoons A_d$$

Thus,

$$\frac{[A_d]}{[A_n]} = K_1$$

and

$$[A_d] = K_1[A_n] \tag{142}$$

Since $A_0 = A_n + A_d$, Eq. 142 in terms of total and active forms of luciferase may be written

$$[A_0] = [A_n] + K_1[A_n]$$

Or

$$[A_n] = \frac{[A_0]}{1 + K_1} \tag{143}$$

Under optimal conditions of salt concentration, with an excess of glucose and oxygen in the medium, the rate of the luminescent reaction and therefore, the intensity of luminescence (I), is limited primarily by the amount and activity of the enzyme A_n and the amount of luciferin L. The reaction proceeds as if governed by a single specific reaction rate constant k':

$$I = bk'[L][A_n] \tag{144}$$

Here b is a proportionality factor which converts the number of molecules reacting into light intensity I. If $[A_n]$ from Eq. 143 is substituted into Eq. 144 the result is

$$I = \frac{bk'[L][A_0]}{1 + K_1} \tag{145}$$

But

$$k' = \kappa \frac{kT}{h} K^{\ddagger}$$

Substituting this value for k' into Eq. 145 gives

$$I = \frac{b\kappa(kT/h)K^{\ddagger}[L][A_0]}{1 + K_1} \tag{146}$$

Now

$$K^{\ddagger} = e^{-\Delta H^{\ddagger}/RT} e^{\Delta S^{\ddagger}/R}$$

and

$$K_1 = e^{-\Delta H_1/RT} e^{\Delta S_1/R}$$

Therefore Eq. 146 becomes

$$I = \frac{b\kappa(kT/h)e^{-\Delta H^{\ddagger}/RT} e^{\Delta S^{\ddagger}/R}[L][A_0]}{1 + e^{-\Delta H_1/RT} e^{\Delta S_1/R}} \tag{147}$$

Equation 147 applies to a given reaction where the net rate is proportional to native enzyme. In living cells it is often difficult or impossible to ascertain the concentration of the reactants, i.e., enzyme and substrate, and $\Delta S^{\ddagger}$, therefore, cannot be readily separated from the concentration factor. It may be considered constant, however, and under steady state conditions the amount of substrate may likewise be considered constant. These unknown constants may then be lumped to-

gether with the proportionality constant b, the transmission coefficient κ, and the known constants k and h, as a single factor c. Hence,

$$I = \frac{cTe^{-\Delta H^{\ddagger}/RT}}{1 + e^{-\Delta H_1/RT}e^{\Delta S_1/R}} \tag{148}$$

Equation 148 is in a form that may be applied to data with respect to the observed rates of reactions catalyzed by a single enzyme as well as complex physiological processes.

In physiological processes, however, it is generally found that the above theory is over simplified, and that reactions in addition to those assumed also influence the observed rate, with the result that the experimental data cannot be fully accounted for on the basis of Eq. 148 above. Because enzymes are complex molecules, temperature and pressure may strongly affect such properties as heat content, entropy, and volume, making the quantities H, S, and V and the analogous quantities for activation strongly temperature and pressure dependent. The temperature dependence may be seen by Eq. 148 directly, keeping in mind that the value of the constant c is also a function of temperature.

To provide a basis for the understanding of the influence of pressure on bacterial luminescence, consider the relation:

$$\Delta F^{\ddagger} = \Delta H_0^{\ddagger} - T\Delta S_0^{\ddagger} + P\Delta V^{\ddagger} \tag{149}$$

Then Eq. 148 can be written:

$$I = \frac{cTe^{-\Delta H_0^{\ddagger}/RT}e^{-P\Delta V^{\ddagger}/RT}e^{\Delta S_0^{\ddagger}/R}}{1 + e^{-\Delta H^0/RT}e^{-P\Delta V/RT}e^{\Delta S_0/R}} \tag{150}$$

The subscript indicates the value of the property at zero pressure. From Eq. 150 it follows that at constant temperature the net effect of pressure will be determined by the values of both $\Delta V^{\ddagger}$ and ΔV. The relative importance of these two terms, however, will vary with temperature, even if the values of $\Delta V^{\ddagger}$ and ΔV do not vary with temperature. In enzyme systems, a large variation in their effect on the over-all rate at different temperatures will occur because the equilibrium constant K_1 for the denaturation reaction of the catalyst is characterized by a high heat and entropy change. At temperatures well below the normal "optimum," therefore, K_1 is negligibly small in comparison to 1, and so has virtually no influence on the rate. The effects of increased pressure then occur practically entirely through changes in the specific rate constant k' of the numerator, other things being the same. Consequently, at low temperature, measurements of the relation between the intensity of luminescence and the amount of pressure provides a means of determining the value of $\Delta V^{\ddagger}$ for the chosen conditions of pH, salt con-

centration, etc. In order to arrive at a value of ΔV, it is desirable to raise the temperature to some point well above the normal optimum, where K_1 is large compared with 1. Here intensity of luminescence is modified by the influence of pressure on both $\Delta V^{\ddagger}$ and ΔV, and measurements of the relation between luminescence intensity and pressure yield the difference of these constants. As a rule, it might be expected that $\Delta V^{\ddagger}$ will be smaller than ΔV, just as the energy and entropy of activation of the enzyme-catalyzed rate process are likely to be much smaller than the heat and entropy of reaction for the reversible denaturation of the protein. For this reason, the process as a whole may be expected to have an optimal pressure, as well as an optimal temperature.

References

1. L. von Bertalannfy, *Science* **111,** 23 (1950).
1a. H. Driesch, "The Science and Philosophy of the Organism." Black, London, 1929.
2. G. N. Lewis and M. Randall, "Thermodynamics." McGraw-Hill, New York, 1923.
3. E. Schrödinger, "What is Life?" Cambridge Univ. Press, London and New York, 1945.
4. N. Wiener, "Cybernetics." Wiley, New York, 1948.
5. E. S. West, "Textbook of Biophysical Chemistry," 2nd ed. Macmillan, New York, 1956.
6. H. A. Krebs and H. L. Kornberg, *Ergeb. Physiol.* **49,** 212 (1957).
7. C. van Niel, *Advances in Enzymol.* **1,** 263 (1941).
8. J. A. Bassham and M. Calvin, "The Path of Carbon in Photosynthesis." Prentice-Hall, New York, 1957.
9. P. Oesper, *Arch. Biochem.* **27,** 255 (1950).
10. T. de Donder and P. van Rysselberge, "Affinity." Stanford University Press, Stanford, California, 1936.
11. I. Prigogine, "Introduction to Thermodynamics of Irreversible Processes." C. C. Thomas, Springfield, Illinois, 1955.
12. L. Onsager, *Phys. Rev.* **37,** 405 (1930).
13. C. Eckart, *Phys. Rev.* **58,** 269 (1940).
14. H. B. C. Casimir, *Revs. Modern Phys.* **17,** 343 (1945).
15. H. B. Callen, *Phys. Rev.* **73,** 1347 (1948).
16. R. T. Cox, *Revs. Modern Phys.* **22,** 238 (1950).
17. R. B. Parlin, R. J. Marcus, and H. Eyring, *Proc. Natl. Acad. Sci. U. S.* **41,** 900 (1955).
18. S. Glasstone, K. Laidler, and H. Eyring, "The Theory of Rate Processes." McGraw-Hill, New York, 1941.
19. C. J. Giddings and H. Eyring, *J. Phys. Chem.* **62,** 305 (1957).
20. B. J. Zwolinski, H. Eyring, and C. Reese, *J. Phys. & Colloid Chem.* **53,** 1426 (1949).
21. H. Eyring and R. Parlin, *Ann. N. Y. Acad. Sci.* **65,** 679 (1957).
22. F. H. Johnson, H. Eyring, and M. J. Polissar, "The Kinetic Basis of Molecular Biology." Wiley, New York, 1954.

CHAPTER 3

Comparative Mechanisms for Fatty Acid Oxidation

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As substrates fatty acids are unique in biological systems since they are the most highly reduced compounds that are completely available as energy sources. Readily synthesized from carbohydrates and stored mainly as triglycerides, on combustion they release more energy than any other general type of foodstuff. For example, on combustion 1 gm. of carbohydrate will yield 4200 cal., 1 gm. of protein, 5600 cal., and 1 gm. of fat, 9300 cal. It is therefore of considerable interest to examine the mechanisms found in organisms for the oxidation of fats and the trapping of large amounts of energy thereby released. No attempt will be made to review the very large literature on the occurrence and structure of fatty acids in different phyla nor their biosynthesis.

I. Energetics of Fatty Acid Oxidation

Since fatty acids are stored mostly as triglycerides, they must first be converted to coenzyme A esters before they can be further utilized. In the hydrolysis of triglycerides to free fatty acids about 2500 cal. of free energy of hydrolysis are liberated in the form of heat, since there is no known mechanism for its conservation. If, however, the ester bonds in triglycerides could be cleaved by a thiolytic split, then the total conservation of the ester linkage would be possible. Since this means the formation of a thioester bond of approximately 8500 cal. of free energy of hydrolysis, it would imply that the equilibrium would be greatly in favor of the synthesis of the ester bond rather than of the thioester linkage. Regardless of the mode of triglyceride cleavage, each fatty acid, free or otherwise, must eventually enter the classic β-oxidative cycle as an acyl CoA derivative. A unique feature of β-oxidation is that a fatty acid must undergo only one initial activation to the energy level of an acyl CoA derivative; thereafter it remains at that energy level as a CoA derivative throughout the complete conversion of the molecule to acetyl-CoA.

It is therefore instructive to examine in detail the energy potential of a fatty acid molecule as it proceeds to acetyl-CoA units. In Table I

TABLE I
HEATS OF COMBUSTION AND FREE ENERGIES OF COMBUSTION OF TYPICAL FATTY ACIDS

Acids	Heat of combustion[a] $\Delta H°$	Free energy of combustion[b] $\Delta F°$
Acetic	209,400 (l)	207,800 (l)
Butyric	524,300 (l)	513,600 (l)
Octanoic	—	1,140,000 (l)
Capric	1,458,100 (s)	1,450,000 (l)
Lauric	1,771,700 (s)	1,750,000 (l)
Myristic	2,085,800 (s)	2,050,000 (l)
Palmitic	2,398,400 (s)	2,339,700 (l)
Stearic	2,711,800 (s)	2,630,000 (l)
Oleic	2,657,000 (l)	—

[a] Calories per gram molecular weight when combustion at atmospheric pressure and 20°. Final products CO_2 (g)· H_2O (l).

[b] Free energy of formation calculated from formula $\Delta F°_{298} = -96{,}000 + 1{,}080\ N$ where molal free energy is in liquid state of normal saturated aliphatic acids containing n carbon atoms (*1*).

are listed both the heat of combustion and the free energy of combustion of a number of fatty acids. If we consider palmitic acid as a typical example,

$$C_{16}H_{32}O_2(s) + 23O_2 \rightarrow 16CO_2 + 16H_2O(l) \qquad (1)$$

$$\Delta F° \text{ of combustion} = -2{,}339{,}700 \text{ cal.}$$

it is now possible to calculate the maximum amount of energy that may be trapped by known mechanisms of β-oxidation in the cell.

Step I:

$$\text{Palmitate} \rightarrow 8 \text{ Acetyl-CoA} + 14 \text{ electron pairs}$$
$$7 \text{ electron pairs} \rightarrow \text{Flavin system } (7 \times 2) = 14 \sim$$
$$7 \text{ electron pairs} \rightarrow \text{DPN}^+ \text{ system } (7 \times 3) = 21 \sim$$
$$\text{Total} = 35 \sim$$

One $\sim$ is required for the activation of palmitate by the ATP-CoA system* and must be deducted from the total synthesized to give 34 $\sim$.

Step II:

$$8 \text{ Acetyl-CoA} + 16 \text{ O}_2 \rightarrow 16 \text{ CO}_2 + 8 \text{ H}_2\text{O} + 8 \text{ CoA}$$

In this step acetyl-CoA is presumed to be degraded through the Krebs cycle to CO_2 and water. Assuming a P/O ratio of 3 for the phosphorylative oxidation of acetyl-CoA, $32 \times 3 = 96 \sim$ are formed. In summation:

$$\text{Step I} = 34 \sim$$
$$\text{Step II} = 96 \sim$$
$$\text{Total} = 130 \sim$$

$$\frac{130 \times 7000}{2{,}339{,}700} \times 100 = 39\%$$

Thus in the complete oxidation of a typical long chain fatty acid such as palmitate, approximately 39% of total available energy is trapped by known systems and suggests the potential importance of fatty acids as sources of useful energy. Whether or not the electron pairs and the thiol ester bonds are channeled as assumed above is however difficult to ascertain.

II. Mechanism for Release of the Potential Energy in Fatty Acids

The calculations made in Section I are based on the classical sequence of β-oxidation. It is therefore worthwhile to examine in some detail the five steps that lead from the initial activation of a fatty acid molecule to the release of an acetyl-CoA unit and then survey the position of this mechanism in other tissues. Figure 1 summarizes the various steps.

* *Abbreviations used in this chapter:* CoA, coenzyme A; AMP, adenosine monophosphate; ADP, adenosine diphosphate; ATP, adenosine triphosphate; GSH, glutathione; P_i, inorganic phosphate; PP, pyrophosphate; DPN^+, diphosphopyridine nucleotide; DPNH, reduced diphosphopyridine nucleotide; ETF, electron transfer flavoprotein; FAD, flavin adenine dinucleotide; TCA, tricarboxylic acid; $\sim$, high-energy bond.

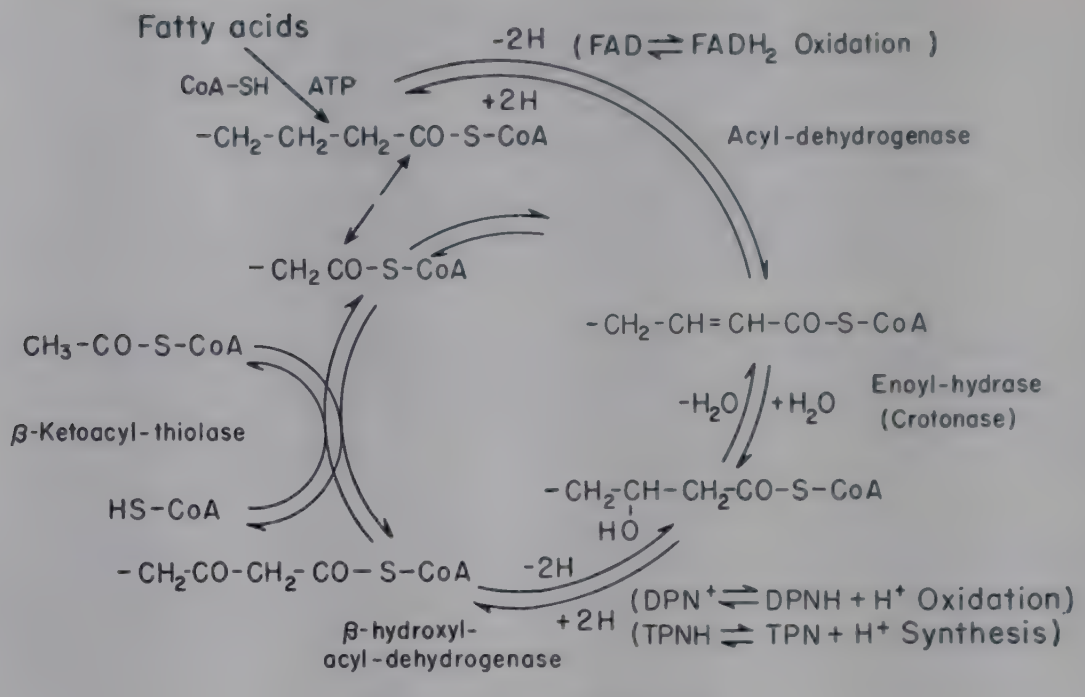


FIG. 1. Fatty acid cycle.

A. Activation Step

The primary activation of fatty acids involves a diphasic reaction:

$$\text{ATP} + \text{RCOOH} \rightleftharpoons \text{AMP-COR} + \text{PP} \quad (2)$$

$$\text{AMP-COR} + \text{CoA} \rightleftharpoons \text{RCO-CoA} + \text{AMP} \quad (3)$$

This type of activation has been found in a wide variety of animal tissues (*2*), in spinach leaf, and mung bean (*3*), avocado, peanut extracts (*4*), yeast (*5*), *Pseudomonas*, and *Rhodospirillum* (*6*). In general there are three types of activities depending on the chain length of the acid. There is the highly specific acetic thiokinase (*5*), and two more general thiokinases, one activating the C_{10}–C_{18} acids including the saturated and unsaturated acids (*7*), and the other activating the C_4–C_{12} acids including the saturated and the 2-ethylenic and 3-ethylenic as well as the α- and β-hydroxyacids (*2*).

Berg (*5*) formulated Reactions 2 and 3 on evidence based on the observations that: (*a*) exchange of P*P* with ATP takes place only in the presence of acetate, (*b*) acetohydroxamic acid forms from ATP and hydroxylamine in absence of CoA, (*c*) synthetic adenyl acetate is converted to ATP in the presence of PP and enzyme, and to acetyl-CoA in the presence of CoA, (*d*) exchange of AMP-C^{14} with ATP is dependent on the presence of acetate and CoA, and (*e*) exchange of acetate-C^{14} with acetyl-CoA requires both AMP and PP.

Since adenyl acylates do not accumulate, they presumably form an enzyme complex according to the reaction

$$\text{ATP} + \text{Acetate} + \text{Enz} \rightleftharpoons \text{Enz-AMP-acetate} + \text{PP} \quad (4)$$

This complex then transfers the acyl group to suitable acceptors such as CoA to form the acylated CoA derivatives.

Acyl adenylates are readily synthetized by reacting acyl anhydrides with AMP in the presence of pyridine as catalyst (*8*). They are relatively stable at acid pH at 0° but are rapidly split at pH 2 at 100°. At pH 10 and above, rapid hydrolysis occurs even at 0°. Acyl adenylates react rather slowly with GSH and CoA to form the acylated thioesters and with amino acids to form acylated amino acids, but the rate of transfer is greatly accelerated by low concentrations of imidazole (*9*). Apparently, acylated AMP transfers its acyl group nonenzymatically to imidazole to form acetyl imidazole and AMP. The catalytic effect of imidazole in the transfer reactions is depicted as

$$\text{AMP-acylate} + \text{Imidazole} \rightarrow \text{AMP} + \text{Acyl-imidazole} \quad (5)$$

$$\text{Acyl-imidazole} + \text{Acceptor} \rightarrow \text{Imidazole} + \text{Acyl-acceptor} \quad (6)$$

At pH 8, the anhydride bond of AMP-acylate has been estimated to have a ΔF° of hydrolysis of about 15,000 cal., indicating that the K of reaction 2 or 4 must be of a low order of magnitude. Since the difference of free energies of hydrolysis between ATP and AMP-acylate is about 8000 cal., the amount of AMP-acylate complex formed would probably be very low. Since the over-all K_{eq} of 2 and 3 or

$$\frac{(\text{acetyl-CoA})(\text{PP})(\text{AMP})}{(\text{acetate})(\text{ATP})(\text{CoA})}$$

is about unity, the tendency to transfer acyl groups from the Enz-AMP-acylate complex to CoA would be great.

Although the thiokinase reaction is probably the main route for acylate activation, there are several indirect pathways which serve an important function in some organisms.

Found in considerable concentration in extracts of *Clostridium kluyveri* (*10*), CoA transphorase catalyzes the reaction:

$$\text{Acetyl-CoA} + \text{Butyrate} \rightleftharpoons \text{Butyryl-CoA} + \text{Acetate} \quad (7)$$

The CoA moiety of acetyl-CoA can be transferred to formate, acetate, propionate, butyrate, valerate, *n*-caproate, *n*-caprylate, vinylacetate, and lactate. The K is about unity. Since the enzyme has not been greatly purified, no specificity can be assigned to a single protein.

A similar transphorase (*11*) has been described for the reaction:

$$\text{Succinyl-CoA} + \text{Acetoacetate} \rightleftharpoons \text{Acetoacetyl-CoA} + \text{Succinate} \quad (8)$$

Succinate is the specific dicarboxylic acid which participates, but several β-keto acids may also be activated. Saturated and unsaturated acids are inert. Since succinyl-CoA is formed in the oxidation of α-

ketoglutaric acid, organisms with TCA cycles potentially can channel the thioester bond energy from succinyl-CoA to the β-keto acid series which then by thiolytic cleavage can yield acetyl-CoA and an activated acyl derivative. At pH 7 in the presence of Mg^{2+}

$$K_{eq} = \frac{(\text{succinate})(\text{acetoacetyl-CoA})}{(\text{succinyl-CoA})(\text{acetoacetic})} = 2.83 \times 10^{-3}$$

The enzyme has been isolated from pig heart, dog skeletal muscle, and ox adrenal glands, but is absent in pigeon breast muscle, rabbit skeletal muscle, and pigeon brain.

In *Clostridium kluyveri*, phosphotransacetylase (*12*) catalyzes the reaction:

$$\text{Acetyl phosphate} + \text{CoA} \rightleftharpoons \text{Acetyl CoA} + \text{Phosphate} \qquad (9)$$

The enzyme is quite specific since the rates of reaction of higher homologs of acetyl phosphate fall off very rapidly.

$$K_{eq} = \frac{(\text{acetyl-CoA})(PO_4)}{(\text{acetyl phosphate})(\text{CoA})} = 74$$

which favors trapping the energy of the phosphoryl carboxylate anhydride bond into the more useful thioester bond. The direct biosynthesis of acetyl phosphate is by means of the acetokinase reaction (*13*):

$$\text{Acetate} + \text{ATP} \rightleftharpoons \text{Acetyl phosphate} + \text{ADP} \qquad (10)$$

The enzyme is highly specific for acetate. The K_{eq} at pH 7 is approximately 0.006 and favors the formation of ATP from acetyl phosphate and ADP. By combining Reactions 9 and 10, the K'_{eq} will be 0.44, which is quite adequate for biosynthetic purposes.

Other sources of acetyl phosphate are:

(*a*) pyruvic oxidase (*14*)—*Lactobacillus delbrueckii*

$$\text{Pyruvic acid} + P_i + O_2 \rightarrow \text{Acetyl phosphate} + CO_2 + H_2O_2$$

(*b*) phosphoroclastic split (*15*)—*Escherichia coli*

$$\text{Pyruvate} + P_i \rightleftharpoons \text{Formate} + \text{Acetyl phosphate}$$

(*c*) phosphoketolase (*16*)—*Lactobacillus pentosus*

$$\text{Xylulose 5-phosphate} + P_i \rightarrow \text{Acetyl phosphate} + \text{Triose phosphate}$$

(*d*) phosphoroclastic split (*17*)—*Clostridium butylicum*

$$\text{Pyruvate} + P_i \rightleftharpoons \text{Acetyl phosphate} + H_2 + CO_2$$

With these systems available for the generation of acetyl phosphate, an organism with phosphotransacetylase can readily transfer the energy of high-energy phosphate to a thioester bond. Acetyl phosphate,

however, plays no function in either plant or animal tissues, since acetokinase and phosphotransacetylase are absent; in bacteria these two enzymes probably play an important role in the formation and utilization of high-energy phosphate.

In many tissues general as well as specific thiol esterases (deacylases) (*18*) are found which carry out the irreversible reaction:

$$\text{RCO-CoA} + \text{H}_2\text{O} \rightarrow \text{RCOOH} + \text{CoA} \qquad (11)$$

The enzymes for the deacylation of succinyl-CoA and acetyl-CoA occur in heart, liver, and bacterial extracts (*19*). It is of some interest to examine the role of these deacylases in tissues. Physiologically, it is known that in liver tissue acetoacetic acid accumulates and is metabolically inert. But in kidney or in heart tissue acetoacetic acid is rapidly consumed. It appears that in liver a significant concentration of an acetoacetyl-CoA deacylase occurs. Moreover β-keto acid thiokinase (*20*) which catalyzes the reaction

$$\text{Acetoacetic acid} + \text{CoA} + \text{ATP} \rightarrow \text{Acetoacetyl-CoA} + \text{AMP} + \text{PP} \qquad (12)$$

is virtually absent in liver but present in kidney and heart (*21*). Once acetoacetic acid is formed by deacylation of its CoA derivative in the liver it becomes metabolically inert. In contrast, in kidney and heart deacylase activity is low and any circulating acetoacetic acid is rapidly channeled by the activating enzymes into the metabolic pool (see Section II,C for further discussion).

It is obvious that the control of the concentration of these enzymes in the cell is of considerable importance in the utilization of acyl CoA derivatives. For example Lynen (*22*) observed that octanoyl-CoA thioesterase in mitochondrial preparations acts as a barrier for the synthesis of long chain fatty acids from acetyl-CoA. Instead of accepting another acetyl unit for long chain fatty acid synthesis octonoyl-CoA is hydrolyzed to the free acid and thus the thioesterase breaks the chain of synthesis reactions. Another type of hydrolytic enzyme is propionyl adenylate hydrolase, which has been found in extracts of an unidentified bacterium isolated from Massachusetts garden soil and grown on propionate as a sole source of carbon (*23*). This enzyme catalyzes the reaction:

$$\text{Propionyl-AMP} + \text{H}_2\text{O} \rightarrow \text{AMP} + \text{Propionate} \qquad (13)$$

In summary, depending on the type of organism, there are several routes for the activation of fatty acids. Once raised to the level of an acyl CoA derivative, no further activation is necessary. One mole of ATP is required for the oxidation of a mole of acid regardless of its

chain length. This is a very favorable condition for the extraction of utilizable energy from fatty acids.

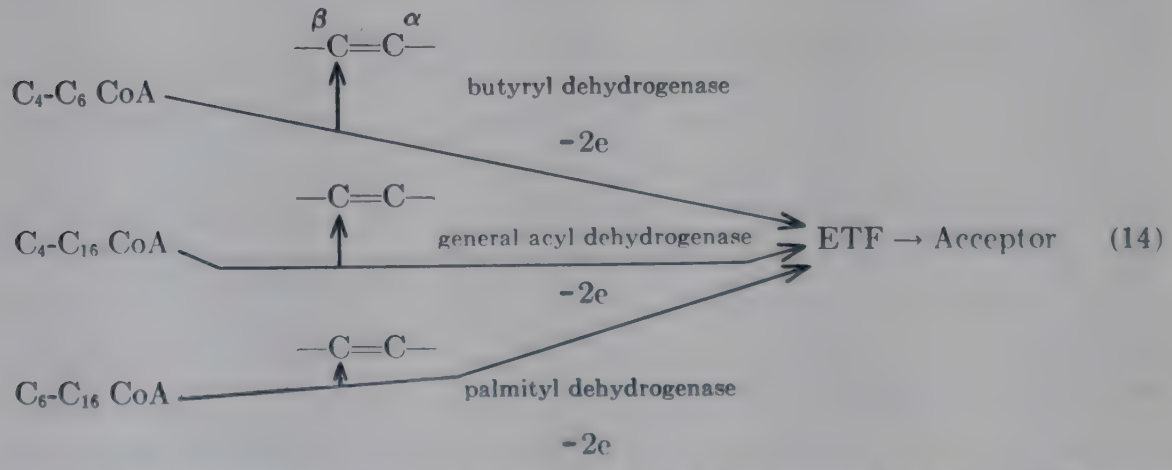
B. Formation of the Ethylenic Group

There are two approaches in the synthesis of the important 2-ethylenic bond in fatty acids: (*a*) by an α-β dehydrogenation, and (*b*) by a dehydration reaction.

1. *Dehydrogenation*

The primary oxidation of acyl CoA involves at least two steps: (*a*) the removal of hydrogen from the α-β carbons of the acyl group by a specific dehydrogenase; and (*b*) the transfer of these hydrogens by a specific flavoprotein, the electron transfer flavoprotein (ETF), to a suitable acceptor. Depending on the substrate specificity, the dehydrogenases are classified as butyryl dehydrogenase (C_4–C_6 acyl CoA) (*24*), general acyl dehydrogenase (C_4–C_{16} acyl CoA) (*25*), and palmityl dehydrogenase (C_6–C_{16} acyl CoA) (*26*).

All the dehydrogenases contain FAD as the prosthetic group and are metal-free. They possess two unique characteristics. First, the reduced flavoproteins are incapable of being reoxidized unless an additional flavoprotein, ETF, is added (*27*). Reduced ETF can then be readily oxidized by a mitochondrial system through cytochrome c. The sequence shown (Reaction 14) summarizes these findings.



Second, an unusually strong enzyme-substrate complex occurs between the acyl dehydrogenases and their respective substrates. Thus the bound substrate cannot be dialyzed, is sedimentable with the protein in the ultracentrifuge, and is precipitable with ammonium sulfate. There is no measurable dissociation of these complexes. In addition, when acyl CoA complexes with its acyl dehydrogenase, both CoA and the acyl moieties are bound as a unit. The bound substrate can be displaced by free substrate of similar affinity to the enzyme, but the substrate molecule

leaves the enzyme as intact acyl CoA. There is no dynamic exchange on the enzyme of CoA and acyl moieties of different molecules. Thus C_{16}-CoA and C_8-CoA (P^{32}) when added to their acyl dehydrogenase do not yield C_{16}-CoA (P^{32}) and C_8-CoA. The molar ratio of substrate to the protein binding is a small number between 1:1 and 2:1, never larger (*28*). The product of oxidation is the α,β-ethylenic group with a *trans* geometric configuration.

With indophenol ($E' = +0.217$ volts) as the oxidant, butyryl-CoA is oxidized practically to completion but with pyocyanine ($E' = -0.034$ volts) an equilibrium constant can be determined. Thus

$$\frac{(\text{pyocyanine} \cdot 2\text{H})(\text{crotonyl-CoA})}{(\text{butyryl-CoA})(\text{pyocyanin})} = 0.25$$

For the couple, butyryl-CoA–crotonyl-CoA (pH 7.0, 30°), an E' of 0.015 volts can be computed (*29*). Of some interest is the comparison of this value with an E' of 0.025 volts calculated by Kalckar (*30*) for butyric-crotonic acids. This would suggest little difference in the yield of free energy by either type of substrate in a thermodynamic sense.

Butyryl dehydrogenase occurs in *Clostridium kluyveri* (*31*) and in mammalian tissues (*24*). It has not been studied extensively in other types of tissues.

Another example of 2-ethylenic dehydrogenation is the reaction catalyzed by the historic succinic dehydrogenase. For many years the nature of the oxidative process remained a mystery, since the enzyme protein was firmly bound to particulate components of the mitochondrial structure. By a combination of new techniques for enzyme extractions and a new assay system employing the appropriate dye, in this case phenazine methosulfate, the enzyme has been fully solubilized, and purified from such diverse material as heart muscle (*32*), yeast (*33*), and *Micrococcus* (*34*).

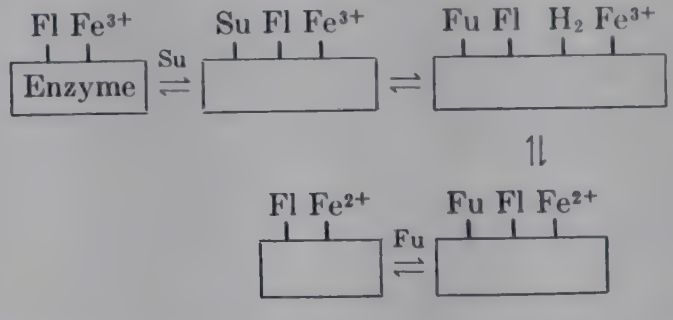
The highly purified enzyme is a ferroflavoprotein with a ratio of iron to flavin of 4:1. The molecular weight is 200,000. The iron moiety is firmly bound to the native protein, since it is not removed by cation resins nor by dialysis against iron chelators. The flavin moiety (Fl) is also firmly associated with the enzyme protein and is liberated from the protein only by controlled tryptic digestion. FAD appears to be held to the apoenzyme by covalent bonds through a peptide linkage presumably at the 3-imino group of the alloxazine ring of the flavin (*34*).

For many years evidence was submitted that a specific enzyme, fumaric reductase, was responsible for the reduction of fumaric acid (Fu) to succinic acid (Su). Singer (*35*) has shown however that if care is taken to select the correct redox dyes for the oxidative and re-

ductive steps, free reversibility can be demonstrated. Thus the ratio

$$\frac{V_{Su}^{25°} \text{ (phenazine methosulfate)}}{V_{Fu} \text{ (FMN} \cdot H^2)}$$

gives a consistent ratio of 8.5. Singer believes that the following mechanism describes in part the sequence of Reaction 15.



(15)

Recent studies indicate that succinic dehydrogenase is nonspecific in distinguishing the hydrogens in the methylene groups in succinic acid (*36*). Thus, heart particle preparations catalyze a slow anaerobic exchange of deuterium from water into succinate. Deuterium in succinate therefore arises directly from the solvent and not from labeled fumarate. Deutero-succinate prepared by anaerobic enzymatic exchange, when oxidized aerobically by the same enzyme preparation, yields fumarate which retains half the isotope content found in the original succinate. Succinic dehydrogenase is absolutely specific. Only two substrates, succinic and fumaric acids, react with the enzyme. It should be mentioned that fumarate is the *trans* geometric isomer. Maleic acid, the *cis* isomer, is completely inert.

2. *Hydration-Dehydration*

The reversible addition or removal of the elements of water from an ethylenic system is catalyzed by enoyl hydrase in the case of monocarboxylic thioesters and by such specific enzymes as fumarase for the fumaric $\leftrightarrow$ malic system and aconitase for the citric $\leftrightarrow$ *cis*-aconitate $\leftrightarrow$ isocitrate system.

In general, the substrates for enoyl hydrase are either 2-ethylenic acyl CoA derivatives of the *trans* configuration or the L(+)-β-hydroxyacyl CoA derivatives. Fumarase similarly reversibly adds water across the double bond system of fumaric, which has a *trans* configuration, to yield malic acid with the L configuration. However, in the case of aconitase, *cis*-aconitic acid is the geometric isomer that is reactive; the *trans* isomer is inert.

a. Enoyl Hydrase. This enzyme, frequently called crotonase, is widespread in many tissues and has been studied extensively by Stern (*37*,

38), who has crystallized it from ox liver extracts. It has a very high turnover number of 1.4×10^6 moles of crotonyl-CoA per minute at 25° for each mole of enzyme. In analyzing the substrate specificities of the enzyme, Stern has shown that the substitution isomers such as ethylenic thioesters with a methyl group in the α- or β-positions greatly decrease the reactivity. The positional isomer, 3-butenoyl-CoA, is hydrated although at a very slow rate (1.5% of the *trans*-crotonyl-CoA derivative). Stern claims that *cis*-crotonyl CoA is hydrated but at a rate one-third that of the *trans* isomer. The *cis* isomer is first converted irreversibly to the *trans* isomer. The *trans* isomer is then hydrated to L(+)-β-hydroxyacyl-CoA. Wakil (*39*) presents strong evidence that crotonase does not have any isomerase activity but does hydrate the *cis* isomer to the D(−) form and the *trans* to the L(+) form. The 4-ethylenic isomer of thioesters is not active. Acrylyl-CoA is hydrated to β-hydroxypropionyl-CoA.

$$K' = \frac{(\text{L(+)-}\beta\text{-hydroxybutyryl-CoA})}{(\text{crotonyl-CoA})(\text{H}_2\text{O})} = 6.18 \times 10^{-2}$$

K' is independent of pH. If $\Delta F' = -RT \ln K' \times 55.5$ and the standard states of solutes is 1 M, then at pH 7.5 and 25° $\Delta F'$ is −730 cal. for the formation of the β-hydroxybutyryl-CoA.

Since crotonase is an SH enzyme, Stern suggests that the reaction mechanism involves the addition of the enzyme across the double bond; the addition complex is broken down by a displacement of the —S-Enz by water to give the hydrated product (Reaction 16).

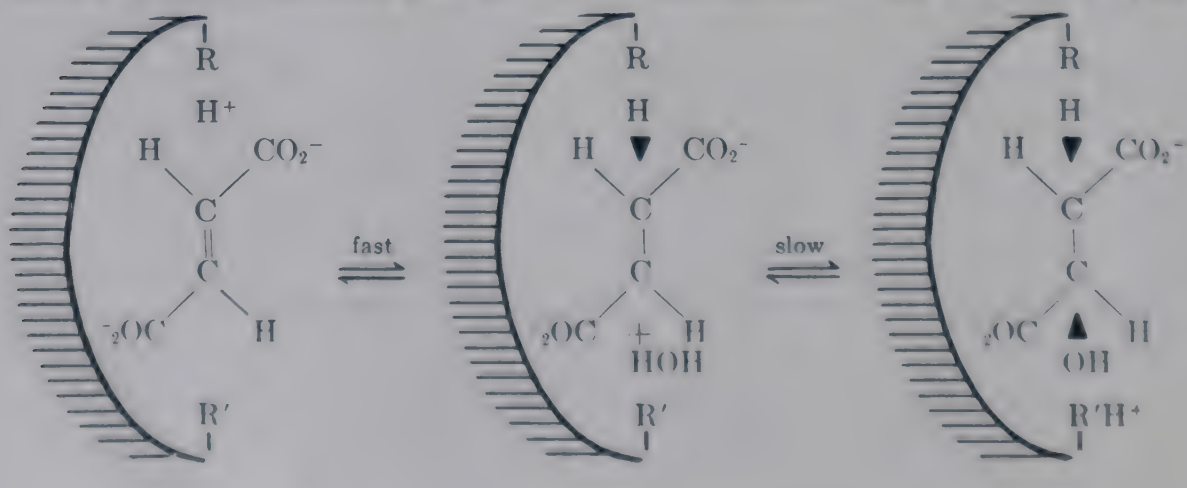
$$\begin{array}{ccc} \text{RCH}_2\text{CH=CHCO-CoA} + \text{HS-Enz} & \rightleftharpoons & \text{RCH}_2\text{CH—CH}_2\text{CO-CoA} \\ & & \quad\quad\quad\

Both 3-ethylenic pentenoyl and 3-ethylenic hexenoyl thioesters are inert. The reaction appears to be irreversible, since crotonyl thioesters do not convert to vinylacetyl thioesters. The equilibrium for the alkali-catalyzed tautomerization of vinylacetic acid to crotonate is greater than 99% in favor of crotonate. This enzyme, first reported in *C. kluyveri,* is also found in *Clostridium tetanomorphum* and in sheep liver homogenate (*31*). If the crystalline crotonase prepared by Stern were contaminated with small amounts of this isomerase, it could explain the low order of activity of crotonase for the 3-ethylenic acyl thioesters which Stern has observed. Crystallinity of an enzyme protein does not guarantee purity.

c. Fumarase. Just as acyl dehydrogenases have as their counterpart succinic dehydrogenase so crotonase has as its counterpart fumarase. Like crotonase, no cofactors have been found associated with the crystalline enzyme. Only two substrates are active, *cis*-fumaric acid and L-malic acid. The enzyme is widespread in different phyla. It has been prepared in the crystalline form and studied extensively.

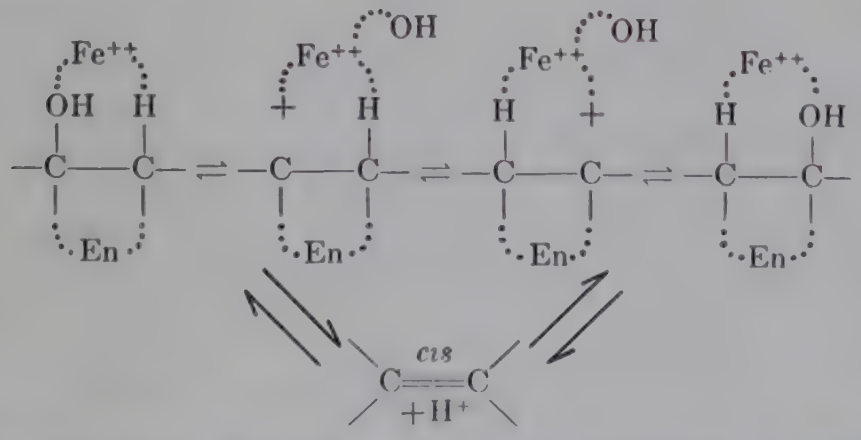
When the hydration of fumaric acid to L-malic acid is catalyzed in D_2O by fumarase, L-malate contains only one deuterium atom per molecule which is not readily exchangeable into the medium (*40, 41*). Since fumarate does not incorporate deuterium, the addition of hydrogen is stereospecific as is the OH incorporation. When monodeuterated malate is dehydrated enzymatically in water, the V_{max} and K_m is the same as for ordinary L-malate. If the breaking of the methylene C—H bond were rate determining, substitution of deuterium for H would produce a decrease in rate. Absence of a deuterium rate effect in the dehydration of monodeutero-L-malate indicates that the exchange of a specific H atom with the H atom of the medium proceeds at a much faster rate than the OH participation. Thus Alberty pictures the reaction as shown (Eq. 18).

$$\text{Enz} + \text{Malic} \rightleftharpoons \text{Enz-malic} \overset{\text{slow}}{\rightleftharpoons} \text{Enz-}X \overset{\text{fast}}{\rightleftharpoons} \text{Enz-fumaric} \rightleftharpoons \text{Enz} + \text{Fumaric} \quad (18)$$



Further evidence based on nuclear magnetic resonance measurements suggests that the addition of water to the ethylenic bond is from a *cis* position or from the same side, presumably from the side nearest the protein, since addition is under stereochemical control (*41*).

d. Aconitase. In contrast to fumarase and crotonase, aconitase has ferrous ion as a participant in the hydration reaction. But like fumarase aconitase demonstrates stereospecificity in the addition and removal of the elements of water across the ethylenic bond. Citrate $\leftrightarrow$ isocitrate conversion involves the transposition of the elements of water, and this may be accomplished by either of two mechanisms: (*a*) removal and addition of water, or (*b*) intramolecular arrangement. By kinetic and deuterium exchange studies (*42*) the second mechanism was found to best describe the aconitase reaction. Thus when isocitrate is incubated with aconitase in deuterium oxide and citrate is isolated, the amount of deuterium incorporated is considerably less than when *cis*-aconitate is the substrate. Further, deuterium oxide inhibits the aconitate-citrate reaction more than it does the isocitrate-citrate reaction. Colowick (*43*) has confirmed these conclusions and has also shown that when deuterium oxide adds across the double bond in *cis*-aconitate, the deuterated citrate or isocitrate which is formed releases the same proton and hydroxyl group as was added originally, since the isolated *cis*-aconitate is now devoid of deuterium. The results have been pictured as shown in Reaction 19.



(19)

It was therefore apparent that in the conversion of citrate to isocitrate, the route is direct and bypasses *cis*-aconitate.

C. Second Dehydrogenation Step

β-hydroxybutyryl dehydrogenase has been obtained in the crystalline form from extracts of pig heart (*44*). It catalyzes the reaction:

$$\text{L(+)-}\beta\text{-Hydroxybutyryl-CoA} + \text{DPN}^+ \rightarrow \beta\text{-Ketobutyryl-CoA} + \text{DPNH} + \text{H}^+ \quad (20)$$

with a turnover number of 42,000 moles of acetoacetoyl-CoA formed

per minute per 100,000 gm. of enzyme at pH 7 and 25°. For the reduction of acetoacetyl-CoA the optimum pH is 6–7; for the oxidation of L(+)-β-hydroxybutyryl-CoA pH 9.6. At the respective pH optima, reduction of acetoacetyl CoA is five times that of the oxidation of β-hydroxybutyryl-CoA. At pH 7 the ratio is 18:1; at 9.6, 1:2.7. The enzyme has a rather broad specificity for substrates. The D(−) isomers are inert. The K_{eq} has been determined at pH 9.6.

$$K'_{eq} = \frac{(\text{acetoacetyl-CoA})(\text{DPNH})(\text{H}^+)}{(\text{L(+)-}\beta\text{-hydroxybutyryl-CoA})(\text{DPN}^+)} = 2.7 \times 10^{-10}$$

Because of the sensitivity of the equilibrium constant and the V_{max} for the hydrogen ion any alteration of the pH in the cell would greatly affect the directions of the reaction and would serve as a site for a control mechanism in the cell.

The D and L forms of β-hydroxybutyric acid have been identified as substrates for some years. These substrates are inert with the β-hydroxyacyl dehydrogenase. However, activating enzymes have been described (*45*) which catalyze the series:

D(−)-β-Hydroxyaliphatic acid + ATP + CoA → D(−)-β-Hydroxyacyl-CoA + AMP + PP (21)

L(+)-β-Hydroxyaliphatic acid + ATP + CoA → L(+)-β-Hydroxyacyl-CoA + AMP + PP (22)

Stern presents evidence that D(−)-β-hydroxybutyryl-CoA is converted to the L isomer by ox liver preparations (*46*). The racemization occurs in the absence of DPN⁺. This racemase has been found in extracts of mitochondria of rat liver, kidney, heart, brain, extracts of *Rhodospirillum rubrum* and *Clostridium acetobutylicum* (*46*). All these also contain the L(+) β-hydroxyacyl dehydrogenase. Wakil pictures the racemization system somewhat differently (*47*). He has observed in beef liver mitochondria a D(−)-β-hydroxyacyl dehydrogenase with DPN⁺ as acceptor and postulates the racemization as follows:

D(−)-β-Hydroxyacyl-CoA + DPN⁺ $\overset{\text{D(+)-dehydrogenase}}{\rightleftharpoons}$ β-Ketoacyl-CoA + DPNH + H⁺ (23)

β-Ketoacyl-CoA + DPNH + H⁺ $\overset{\text{L(+)-dehydrogenase}}{\rightleftharpoons}$ L(+)-β-Hydroxyacyl-CoA (24)

The interconversion of free acetoacetic acid to its reduced forms has several interesting aspects. Thus mitochondria of rat kidney, heart, brain, and pigeon breast muscle oxidize acetoacetic acid, whereas rat liver mitochondria show no activity (*21*). Liver, kidney, and heart mitochondria oxidize both stereoisomers of β-hydroxybutyric acid, whereas brain oxidizes only the D(−) isomer at an appreciable rate. Pigeon breast muscle does not oxidize either isomer of β-hydroxybutyric acid. The several interconversions of acetoacetic acid and its reduced stereoisomers are depicted in Fig. 2.

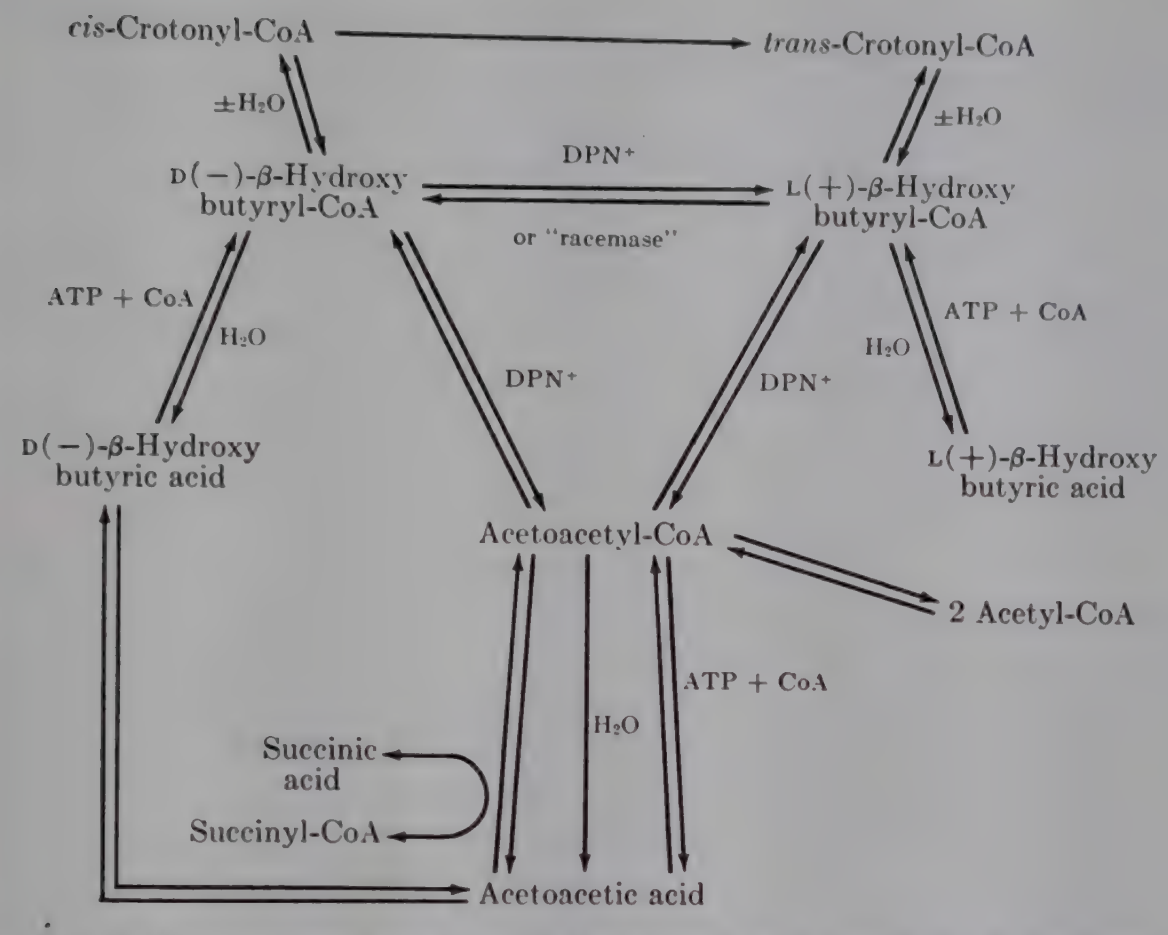


FIG. 2. Interconversion reactions of acetoacetic acid. [After W. P. McCann, *J. Biol. Chem.* **226**, 15–22 (1957)].

D. Thiolytic Cleavage

Thiolase has a twofold importance in the utilization of energy in the degradation of a fatty acid molecule. In the first place, if the β-keto acyl CoA derivative were split by the addition of water, then acetyl-CoA plus a free C_{n-2} aliphatic acid would be formed. This free acid would have to be reactivated by the ATP-CoA thiokinase system. ATP would be required as each C_2-unit is released in order that the breakdown of fatty acids continue. By a thiolytic cleavage, the thioester energy is preserved and the C_{n-2}-residue will remain in the activated stage. In the second place, since the thiolytic cleavage is reversible, it may by this mechanism synthesize a C—C bond. Purified thiolase (*48*) acts rapidly with acetoacetyl-CoA but not with the higher homologs, whereas the crude homogenates react with the higher ketoacyl homologs. At pH 7

$$K_{eq} = \frac{(\text{acetyl-CoA})^2}{(\text{acetoacetyl-CoA})(\text{CoA})} = 3 \times 10^4$$

K may be shifted to the condensation side by raising the pH or by the addition of Mg^{2+}, which forms chelate complexes with β-ketoacyl CoA.

The β-ketoacyl thiolase is an SH enzyme and Lynen (*48*) pictures its action as follows:

$$RCH_2COCH_2CO\text{-}CoA + Enz\text{—}SH \rightleftharpoons RCH_2CO\text{—}S\text{—}Enz + CH_3CO\text{-}CoA \quad (25)$$

$$RCH_2CO\text{—}S\text{—}Enz + CoA \rightleftharpoons RCH_2CO\text{-}CoA + Enz\text{—}SH \quad (26)$$

The HS-Enz acts as the base reagent and leads the initial attack on the β-keto substrate. CoA appears at the end of the reaction to displace the S-enzyme from the acyl-S-enzyme complex. Evidence suggests that CoA is not the initial component involved in the cleavage reaction.

Thiolase has been found in a wide variety of mammalian organs (although low in brain), and in *Clostridium kluyveri, C. saccharobutyricum,* and *C. butyrium* (*31*).

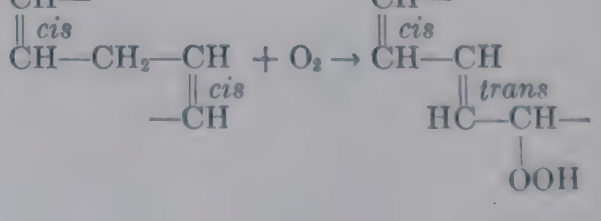
In summary, five distinct steps are required to cleave acetyl-CoA from a long-chain fatty acid. Two steps are oxidative; presumably the electrons derived from these steps can be channeled into oxidative phosphorylation sequence for a conservation of energy. However, the primary extraction of energy is in the complete combustion of acetyl-CoA via the Krebs cycle. For most organisms the entire series of five reactions takes place in the mitochondrial particle. There, presumably, the enzymes are spatially arranged in a configuration adjacent to the structures associated with the cytochrome systems and the site of oxidative phosphorylation. It is to be supposed that such an arrangement constitutes a highly efficient system for the production and channeling of high-energy bonds.

III. Non-Energy-Yielding Systems

Despite the prevalence of the classic β-oxidative system that is so beautifully geared to trap and conserve energy, in some plants there are two systems that display highly specific reactions with long chain fatty acids but do not appear to function in the channeling of the reaction into useful work. These are lipoxidase and fatty acid peroxidase.

A. Lipoxidase

This enzyme attacks methylene-interrupted unsaturated fatty acids in which the double bonds have a *cis*-configuration. This oxidative system forms hydroperoxides. Linoleate and linolenate are typical substrates; oleate is inert.



(27)

It is found in many plants, and there have been reports of its existence in animal tissues (*49*). Soybean lipoxidase has been obtained in crystalline form and has a molecular weight of 102,000. The amino acid composition of the crystalline protein shows no unusual amino acids; nor does it contain any metal or non-amino structure in significant concentration (*50*). However, recently urd bean and mung bean lipoxidase were shown to have essential sulfhydryl groups although all other lipoxidases do not (*51*). Tappel has proposed a mechanism of lipoxidase-catalyzed linoleate oxidation which is in harmony with free-radical mechanisms and with the established theory of the oxidation of 1,4-diolefins (*49*). Franke has collected a thorough review of the information on this enzyme and should be consulted for further information (*52*).

The role of lipoxidase in the plant cell is at present poorly understood. There is some indirect evidence that the protein, serving as a temporary electron sink, holds momentarily an electron derived from the double bond flanked methylene group of linoleate or linolenate. In an *in vitro* system consisting of crystalline lipoxidase and substrate, the electron which is abstracted temporarily must of necessity be returned to the free radical substrate to yield the hydroperoxide. Perhaps in the cell this electron may be channeled to conventional electron transport systems, while the highly reactive free radical that has been formed may then initiate reactions in which free radicals participate.

B. Fatty Acid Peroxidase

This enzyme has been found in extracts of cotyledons from germinated peanut seedlings and catalyzes the reaction (*53*):

$$RCH_2CH_2COOH + 2\ H_2O_2 \rightarrow RCH_2CHO + CO_2 + 3\ H_2O \qquad (28)$$

There is good presumptive evidence that the main reaction products are carbon dioxide and a long-chain aldehyde with a chain length one carbon less than its precursor fatty acid. The reaction requires the generation of hydrogen peroxide by such systems as the glycolic acid-glycolic oxidase system. The system is sensitive to azide in low concentrations and cyanide at higher concentration, which would suggest the involvement of a porphyrin-metal complex in the catalytic reaction. It will remove only one carbon, namely the carboxyl carbon of either a long-chain mono unsaturated or a saturated fatty acid. Further oxidation in the hydrocarbon chain does not occur. However, a microsomal system from peanuts in the presence of DPN^+ can release C-2 and C-3 carbons as CO_2 at decreasing rates, thereby suggesting a stepwise α-oxidation. Recent work with extracts of acetone powders of peanut

seedlings has unified these previous observations into a system whereby a fatty acid (C_n) is first peroxidatively decarboxylated into the C_{n-1} aldehyde and CO_2. The aldehyde is oxidized by a DPN specific dehydrogenase to a C_{n-1} acid which then undergoes a repeat of the cycle. This is depicted in Fig. 3.

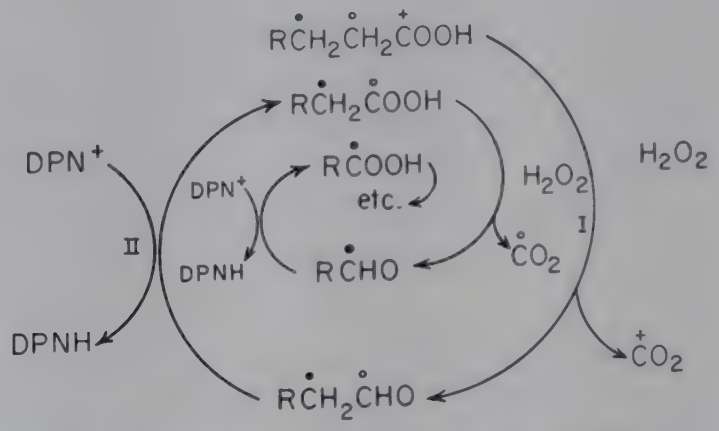


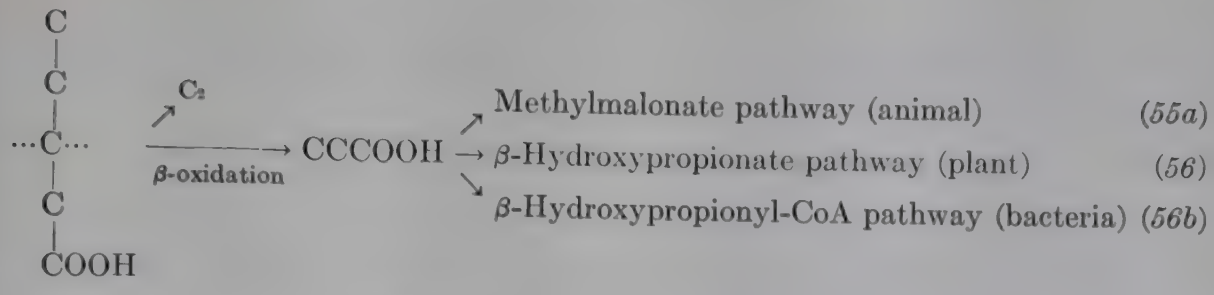
FIG. 3. α-Oxidation of long-chain fatty acids. KEY: (I) long-chain fatty acid peroxidase; (II) aldehyde dehydrogenase.

That the peroxidase oxidation plays a definite role in fatty acid metabolism in plants is indicated by the observation that in the release of carboxyl carbon in a mitochondrial preparation half of the total oxidation is catalyzed by the peroxidase while the remainder is related to the classic β-oxidation system. The role of the system in the cell is however obscure. Fatty aldehydes occur as even carbon chain compounds and are probably formed by the reduction of a thioester by an enzyme similar to that of Burtom and Stadtman which reduces acetyl-CoA to acetaldehyde (*54*).

In both these systems there is no conventional method for trapping the available energy. Further research in this area should bring into sharper focus the role of these systems in the plant cell.

IV. Mechanism of Odd-Chain Fatty Acid Oxidation

Although propionic acid undergoes several transformations in the cell, for several years investigators thought that the primary attack was by α-oxidation via the sequence propionate → acrylate → lactate → pyruvate. No real support for this reaction sequence could be furnished. In fact, much evidence from sheep rumen organisms, propionic acid bacteria, *Micrococcus lactilyticus*, and green photosynthesizing bacteria indicated a close relationship between succinate and propionic acid and carbon dioxide (*55*). The metabolism of odd-chain fatty acids can now be described in rather precise terms and reveals three distinct pathways:



A. Methylmalonate Pathway

Propionate is oxidized in animal tissues by the following unique pathway:

(i). Propionate + ATP + CoA $\xrightarrow{Mg^+}$ Propionyl CoA + AMP + PP (Propionic kinase)

(ii). Propionyl CoA + HCO_3^- + ATP $\xrightarrow{Mg^+}$ Methylmalonyl-CoA + P_i + ADP (Propionyl-CoA carboxylase)

(iii). Methylmalonyl-CoA → Succinyl-CoA (Methylmalonyl CoA isomerase)

This pathway completely explains the experiments of Wood, who showed that when labeled propionate was injected into a fasting rat either α or β carbon atoms of propionate were equally distributed between carbons 1, 2, 5, and 6 of liver glucose.

B. β-Hydroxypropionate Pathway

In several plant mitochondrial systems, propionate is readily oxidized by a modified β-oxidative pathway via β-hydroxypropionate (*56*). The evidence supports the scheme:

(i). Propionate + CoA + ATP → Propionyl-CoA + AMP + PP

(ii). Propionyl-CoA $\xrightarrow{-2H}$ Acrylyl-CoA

(iii). Acrylyl-CoA + H_2O → β-Hydroxypropionyl-CoA

(iv). β-Hydroxypropionyl-CoA + H_2O → β-Hydroxypropionate + CoA

(v). β-Hydroxypropionate $\xrightarrow{-2H}$ Malonic semialdehyde

(vi). Malonic semialdehyde + CoA $\xrightarrow{-2H}$ Malonyl-CoA

(vii). Malonyl-CoA → Acetyl-CoA + CO_2

Carbon dioxide is rapidly released exclusively from the carboxyl of propionate. The methyl and carboxyl groups of acetyl-CoA are derived from carbon two and three of propionate respectively. No evidence is available to support either the pyruvate or methylmalonate pathways in plant tissues. Of considerable interest are the observations of Rendina and Coon (*56a*), who have observed that in many animals tissues Reactions (i)–(v) can occur. Malonic semialdehyde is then aminated by a transaminase in the presence of glutamic acid to yield β-alanine. Evi-

dently Reaction 6 is absent in animal tissues, since there is no evidence of propionic acid undergoing this complete sequence [Reactions (i)-(vii)].

C. Bacterial Pathway (*56b*)

Oxidation of propionate by extracts of *C. kluyveri* involves the following series of reactions:

(i). Acetyl phosphate + CoA ⇄ Acetyl-CoA + P_i

(ii). Acetyl-CoA + Propionate ⇄ Propionyl-CoA + Acetate

(iii). Propionyl-CoA ⇄ Acrylyl-CoA + 2 H

(iv). Acrylyl-CoA + H_2O ⇄ β-Hydroxypropionyl-CoA

(v). β-Hydroxypropionyl-CoA + TPN ⇄ Malonyl semialdehyde-CoA + TPNH + H

(vi). Malonyl semialdehyde-CoA + TPN ⇄ Malonyl-CoA + TPNH + H

(vii). Malonyl-CoA ⇄ Acetyl-CoA + CO_2

Note that while this series of reactions resembles the plant system, the most important differences are Reactions (i), (ii), and (v). In plant material Reaction (iv), the deacylation of β-hydroxypropionyl-CoA, is essential. In addition acetyl-CoA enters the Krebs cycle pathway, whereas in the *C. kluyveri* system it undergoes condensation presumably to higher fatty acids or serves to activate other fatty acid by Reaction (ii).

Reactions (i) to (iii) also occur in extracts of *C. propionicum*. In addition β-alanine-CoA is formed by the amination of β-acrylyl-CoA in the presence of a specific enzyme, acrylyl-CoA aminase.

V. Fatty Acid Oxidation — Comparative Aspects

A. Anaerobic Bacteria

The remarkable ubiquity of certain metabolic patters has been elegantly demonstrated by the studies of fatty acid metabolism in the anaerobic bacterium, *Clostridium kluyveri*. This organism, isolated from the mud of a lake bottom (57), is an obligate anaerobe which secures energy for its growth through the oxidation of ethanol, certainly an aberrant way of life by ordinary standards. Yet the study of this obscure species has provided much of the basis of the understanding of fat metabolism throughout the plant and animal kingdoms.

The over-all conversions effected by *C. kluyveri* were worked out by Bornstein and Barker a number of years ago (58, 59), and the details of those conversions have been investigated intensively since then. The

basic equation of the energy-yielding reactions of the bacterium is as follows:

$$\text{Ethanol} + \text{Acetate} \rightarrow \text{Butyrate} + \text{Caproate} + \text{Acetate} + H_2 + H_2O \qquad (29)$$

Ethanol is specifically required for the fermentation, but acetate can be replaced by propionate, in which case the end products are valeric and heptanoic acids, 5- and 7-carbon compounds respectively. The organisms must be supplied with CO_2, nutrient salts, and minute quantities of biotin and *p*-aminobenzoic acid. Air must be excluded completely from the cultures. A small amount of hydrogen is produced from ethanol by the reaction:

$$\text{Ethanol} + H_2O \rightarrow \text{Acetic acid} + 2\ H_2 \qquad (30)$$

However, about 90% of the ethanol metabolized is accounted for by its conversion to acetate and ultimately to longer chain fatty acids. This latter process is an oxidation-reduction reaction in which ethanol is oxidized to acetate, the acetate formed condenses with other acetate molecules, and the intermediates of the condensation are reduced to butyric and caproic acids through the reducing power of the original ethanol oxidation. These are the only energy-yielding reactions available to *C. kluyveri* (see also Fig. 4).

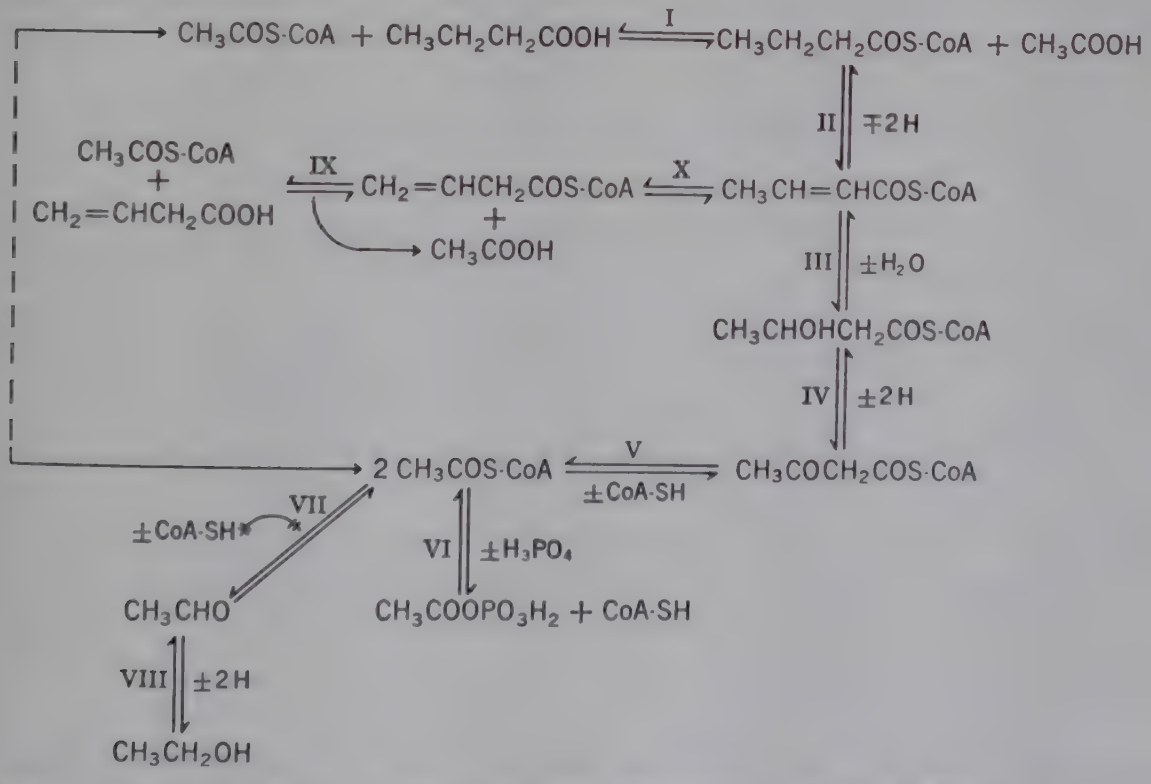


FIG. 4. Scheme of fatty acid synthesis in *Clostridium kluyveri*. Enzyme systems implicated in the above reactions: (I) CoA-S-transphorase; (II) butyryl dehydrogenase; (III) enoyl hydrase; (IV) β-hydroxyacyl dehydrogenase; (V) β-ketoacyl thiolase; (VI) phosphotransacetylase; (VII) aldehyde dehydrogenase; (VIII) alcohol dehydrogenase; (IX) CoA-S-transphorase; (X) vinylacetyl isomerase.

At the time these investigations were being made Rittenberg and Bloch (*60*), using isotopically labeled compounds, had already shown that fatty acid synthesis proceeds by the condensation of active two carbon derivatives of acetate. Barker then realized the peculiar advantages of *C. kluyveri* as a test organism in the study of fatty acid metabolism, since its fatty acid interconversions are uncomplicated by the ramifications of carbohydrate metabolism. The subsequent work was carried out by a number of researchers, most notably E. R. Stadtman and H. A. Barker.

Their research was substantially aided by the discovery that cell-free extracts of the organism were quite active and stable. Further, the somewhat bizarre observation was made that those extracts possessed an active terminal oxidase system which made possible the oxidation of butyric and caproic acids by molecular oxygen (*61*). Since the final electron acceptor under those conditions was oxygen, the intermediates in fatty acid synthesis, i.e., the normal electron acceptors, were not reduced and tended to accumulate in the medium.

In 1951 Lynen and Reichert (*62*) isolated the active derivative of acetate, acetyl-S-CoA, from yeast extracts. Until then the presence of such a compound had been inferred, but its actual discovery made it possible to clarify most of the obscure points remaining in this problem.

Figure 4 shows the scheme for the mechanism of fatty acid synthesis in *C. kluyveri* presented by Barker and later modified by Stadtman (*63*).

The interconversions of this scheme differ from those in animals, aerobic bacteria, and presumably higher plants by the presence of the enzymes phosphotransacetylase, vinylacetylisomerase, and CoA-S-transphorase and by the presence of the intermediate compound, acetyl phosphate, resulting from the activity of phosphotransacetylase. The lack of CoA-S-transphorase prevents other organisms from catalyzing the direct transfer of CoA groups from one fatty acid to another. On the other hand, animals, plants, and aerobic bacteria possess the tricarboxylic acid cycle through which CoA-SH is regenerated as a result of the condensation of acetyl-S-CoA and oxaloacetate to citrate, and ATP is produced by oxidative phosphorylation.

The nature of the mechanisms by which *C. kluyveri* obtains energy from the conversion of ethanol and acetate to butyrate and caproate is not known. In the oxidation of ethanol, acetyl-CoA is formed and the energy of the thioester bond is available to form ATP:

$$\text{Acetyl-CoA} + \text{P}_i \rightarrow \text{Acetyl phosphate} + \text{CoA} \quad \text{(phosphotransacetylase)} \qquad (34)$$

$$\text{Acetyl phosphate} + \text{ADP} \rightarrow \text{Acetate} + \text{ATP} \quad \text{(acetokinase)} \qquad (35)$$

However, acetyl-CoA is the precursor of butyrate and caproate and also is used to form the electron acceptors, acetoacetyl-CoA and crotonyl-CoA, without which ethanol and acetaldehyde cannot be oxidized. Another potential mechanism that has been considered by Barker is the coupling of an electron transport reaction with phosphorylation. Thus the ethanol-aldehyde and crotonyl-CoA–butyryl-CoA system ($\Delta F' = -18$ kcal.) with a simultaneous coupling of phosphorylation has sufficient energy capacity in theory but has never been demonstrated.

Recently Shuster and Gunsalus (*64*) have re-examined the *C. kluyveri* system (crotonyl-CoA + $H_2 \rightarrow$ butyryl-CoA) and have noted a formation of high-energy phosphate coupled with the electron transport process. Approximately 1 mole of ATP is detected for each mole of 2-alkenoic thioester reduced. P_i is esterified to ADP to form ATP.

The aerobic oxidation of butyrate has been studied with extracts of two other *Clostridia, C. saccharobutyricum* and *C. acetobutylicum*. Their metabolic patterns have been found to be similar, if not identical, with those of *C. kluyveri* (*64a*).

β-Oxidation has also been observed in the fatty acid metabolism of the methane bacteria, another anaerobic group, with certain variations of the fundamental scheme. In fact, the first demonstration of the biochemical conversion of higher fatty acids to acetate was shown by Barker to occur in a member of this group (*65*). Energy is obtained by these organisms through the oxidation of fatty acids to acetate and the concomitant reduction of CO_2 to methane. One species, *Methanobacterium propionicum*, oxidizes propionic acid at the alpha carbon, decarboxylates the intermediate formed, and reduces the CO_2 evolved to methane (*66*).

$$\textit{Oxidation:}\quad 4CH_3CH_2COOH + 8H_2O \rightarrow 4CH_3COOH + 4CO_2 + 24H \tag{33}$$

$$\textit{Reduction:}\quad 3CO_2 + 24H \rightarrow 3CH_4 + 6H_2O \tag{34}$$

$$\textit{Observed:}\quad 4CH_3CH_2COOH + 2H_2O \rightarrow 4CH_3COOH + CO_23CH_4 \tag{35}$$

Limited β-oxidation occurs in *M. suboxydans*, which converts valeric to propionic and acetic acids and in a similar manner oxidizes butyric and caproic acids to acetate (*66*) with the reduction of 1 mole of CO_2 to methane:

$$2CH_3CH_2CH_2COOH + 2H_2O + CO_2 \rightarrow 4CH_3COOH + CH_4 \tag{36}$$

Other groups of anaerobes also utilize fatty acids as reducing agents in energy-yielding reactions. Oxidants vary according to the genus and species (*67*). It should be emphasized that in these cases just discussed the fatty acids act as reducing agents rather than oxidants, as in *C. kluyveri*, but growth is still under anaerobic conditions.

B. Aerobic Bacteria

Enzymes concerned with fatty acid oxidation are primarily constitutive, since cells not previously in contact with fatty acid substrates show a high level of metabolism (*68*). Some strains of *Serratia marcescens* (Alphin) and *Bacillus brevis* suggest formation of adaptive enzymes because of the lag period in the oxidation of C_{18} acid by the organism grown on glucose and the elimination of the lag when the organism is grown in a caproate medium. Resting cell suspensions of several strains of *Pseudomonas, E. coli, B. brevis, B. firmis, B. subtilis,* and *B. megatherium* readily oxidized capric and pelargonic acids.

Among the aerobic and facultative bacteria studied, β-oxidation of fatty acids to active acetate fragments again appears to be the principle mechanism of their degradation.

In an investigation of *Mycobacterium tuberculosis*, Iida (*69*) found that saturated fatty acids with even-numbered straight carbon chains are oxidized by constitutive enzymes, and the acids with odd-numbered straight chains by adaptive enzymes. By paper chromatography he demonstrated that caproate (C_6) was an oxidation product of caprylate (C_8) supporting the concept of β-oxidation.

More direct evidence was provided by use of cell-free extracts of *Pseudomonas fluorescens* (*70*). These extracts catalyzed the formation of acetate from decanoate with the uptake of 1 μmole of O_2 per μmole of substrate. Acetyl-S-CoA participation was inferred from the formation of an acetyl hydroxamate upon incubation of the reaction mixtures with hydroxylamine. By submitting the crude extract to an anion exchange resin a requirement for the cofactors ATP, CoA, and Mg^{2+} was demonstrated.

Murray and Dawes (*71*) established that fresh and freeze-dried cells of *Sarcina lutea* oxidize C_2, C_4, C_5, C_6, C_8, and C_{10} fatty acids. Specific enzymes shown to be active in cell-free extracts included aceto-CoA kinase, butyryl dehydrogenase, and acyl dehydrogenase.

The existence of the β-hydroxy intermediate in fatty acid oxidation by aerobic bacteria was demonstrated in *Nocardia opaca*, a soil Actinomycete (*72*). Incubation of the cells with the substrate γ-(3-chlorophenoxy)-butyric acid gave the corresponding acetic acid but much larger quantities of the β-hydroxybutyric acid derivative.

Additional evidence for the general nature of these oxidative pathways was provided by a study of a species of *Vibrio* (*73*). With cell-free extracts of that organism octanoate is oxidized by molecular oxygen with an accumulation of acetic acid. DPN, CoA, and Mg^{2+} are required

cofactors. Subsequent oxidation of the acetate units is by way of the tricarboxylic acid cycle.

The enzyme phosphotransacetylase has not been found in any of the aerobes studied so far. Presumably in this group acetate is activated by ATP and CoA and passed through the tricarboxylic acid cycle as it is in the tissue of animals and higher plants.

C. Fungi

Fatty acid oxidation in the fungi has not been studied as extensively as it has been in bacteria and animals. However, in those species of *Aspergillus* and *Penicillium* which have been investigated, β-oxidation seems to comprise the major degradative pathway (Fig. 5). Character-

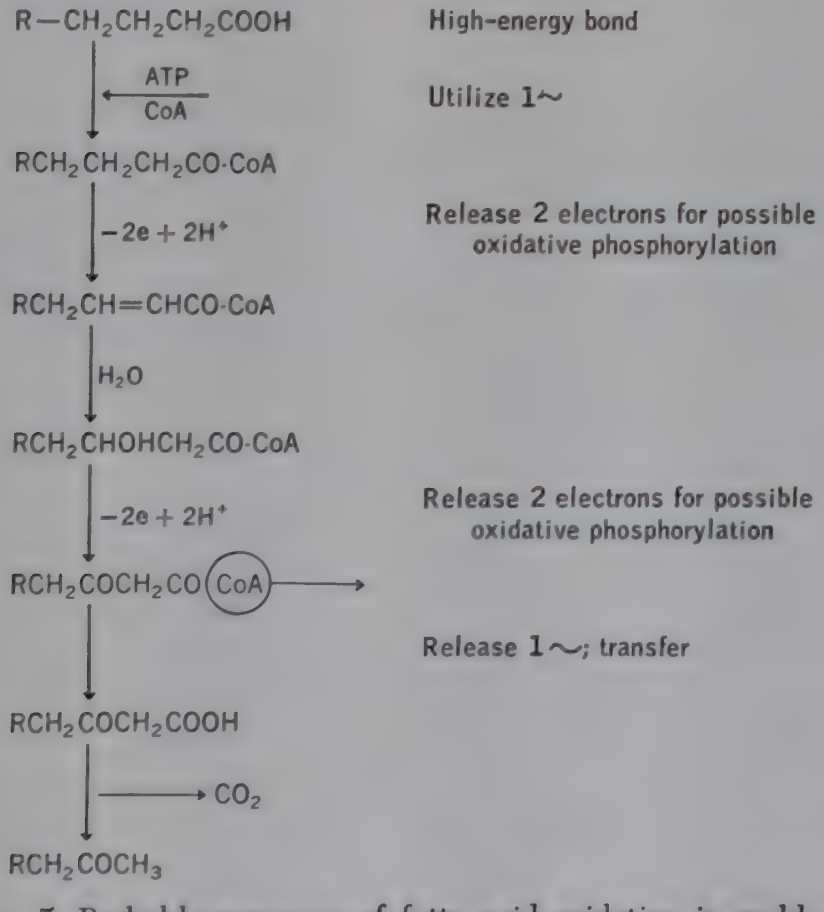


Fig. 5. Probable sequence of fatty acid oxidation in molds.

istic of oxidations in this group is the production of methyl ketones (*74, 75, 76*). This implies an incomplete oxidation which must deprive the cell of some of the energy ordinarily derived from the complete oxidation of such substrates. The oxidation apparently occurs as follows:

$$RCH_2CH_2COOH + O_2 \rightarrow R\overset{\overset{O}{\|}}{C}CH_3 + CO_2 + H_2O \qquad (37)$$

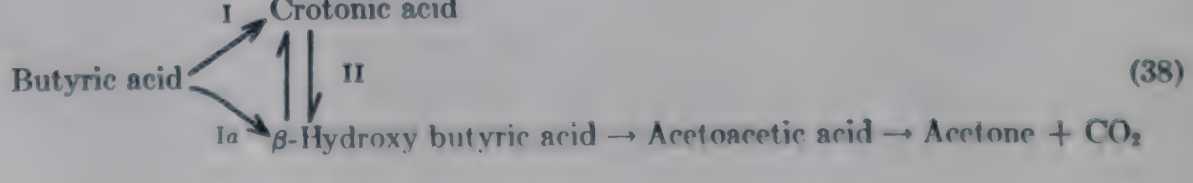
For example, *Penicillium glaucum*, *Aspergillus niger*, and *A. fumigatus*, when incubated with the indicated substrates, give some of the methyl ketones shown in the tabulation.

BREAKDOWN OF FATTY ACIDS TO CORRESPONDING METHYL KETONES BY MOLDS

Fatty acids	Methyl ketones
Butyric acid (C_4)	Acetone
Isovaleric acid (C_5)	Acetone
Valeric acid (C_5)	Methylethylketone
Caproic acid (C_6)	Methylpropylketone
Heptanoic acid (C_7)	Methylbutylketone
Caprylic acid (C_8)	Methylamylketone
Pelargonic acid (C_9)	Methylhexylketone
Capric acid (C_{10})	Methylheptylketone
Undecylic acid (C_{11})	Methyloctylketone
Lauric acid (C_{12})	Methylnonylketone
Myristic acid (C_{14})	Methylundecylketone

In the course of the original observations of methyl ketone formation (*74*) a carbon-chain length optimum of C_8 to C_{12} in the fatty acid substrate was indicated. It was discovered later, however, that the optimum chain length is influenced by the strain used and also by the conditions of growth (*74*). Recently (*75*) it has been shown that mycelial suspensions of *Penicillium roqueforti* can produce methyl ketones from fatty acids with from 4- to 12-carbon chains. The longer fatty acids, palmitic, stearic, and oleic, have never been found to be active substrates in these fungi. These acids tend to exert toxic effects on the organism.

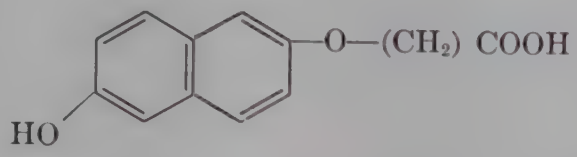
Very little is known as yet about the intermediates of these reactions or of the enzymes involved. β-Unsaturated and β-hydroxy fatty acids serve as substrates for methyl ketone formation in *Penicillium glaucum* (*74*), suggesting their participation in the oxidative process. Mukherjee (*76*) using growing cultures and mycelial suspensions of an *Aspergillus* species found that butyric, crotonic, and β-hydroxy butyric acids supplied as substrates gave rise to acetoacetate or acetone. Butyric acid also yields a small amount of crotonic and β-hydroxy butyric acids. In the presence of HCN the oxidation of substrate crotonic acid was inhibited completely, butyrate was inhibited 46%, and the oxidation of β-hydroxy butyrate was not inhibited at all. In addition, HCN enhanced the accumulation of crotonic acid when butyrate was employed as substrate. Mukherjee proposes the following scheme (Eq. 38) to explain this behavior.



(38)

Reaction I*a* (in Eq. 38) is without analogy. Most probably CoA-S-derivatives of these compounds are involved in the system. The interpretation given to the results is therefore in question, since only the free acids have been tested as substrates.

Recently Byrde and associates (77) supplied ω-(2-naphthoxy) fatty acid substrates to mycelial mats of *Aspergillus niger*. The nature of the oxidation products separated by paper chromatography indicated the substituted fatty acids had been degraded by oxidation at the β position. In the course of this oxidation an OH group was also formed at position 6 of the molecule:



D. Higher Plants and Animal Tissues

In higher plants reference has already been made to the several mechanisms available to the plant cell for the oxidation of fatty acids. The roles of lipoxidase and fatty acid peroxidase are not clear at this time. In addition several types of dehydrogenases for free fatty acids have been described, although complete characterization of these en-

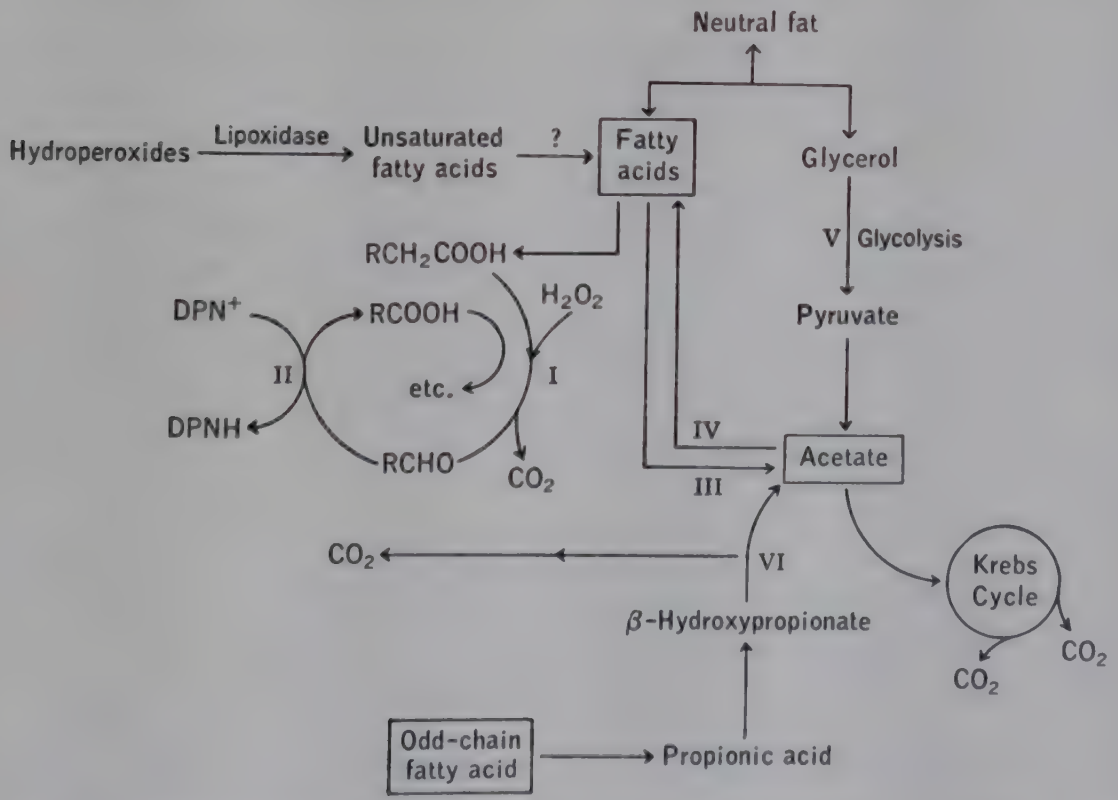


FIG. 6. Interconversion of fatty acids in higher plants. KEY: (I) fatty acid peroxidase; (II) aldehyde dehydrogenase; (III) β-oxidative pathway; (IV) synthetic system; (V) glycerol → pyruvate system; (VI) β-hydroxypropionate pathway.

zymes is not available (52). The classic β-oxidation sequence of fatty acid oxidation has been shown to occur in peanut mitochondria (78). In the presence of CoA, ATP, Mn, a Krebs cycle acid, DPN, TPN, and GSH, a long series of fatty acids is readily attacked and broken down by the classical sequence. In higher plants there are therefore several sequences for handling fatty acids; information on the orderly control of these sequences is therefore of considerable importance. Fig. 6 summarizes this picture.

In animal tissues the dominating pathway is of course the β-oxidative sequence. This unusual mechanism readily explains the properties of long-chain fatty acids as unique sources of energy for the requirements of the cell. Recent reviews have summarized the many papers on this subject (79).

VI. Conclusion

The development of the field of lipid oxidation closely paralleled the growth of modern biochemistry. The early works of Knoop outlined the concept of β-oxidation by feeding appropriate derivatives of fatty acid to whole animals. With the advent of radioisotopes a new period of investigation was initiated by Schoenheimer in the late thirties. Leloir was the first investigator in 1940 to prepare a homogenate which was capable of limited oxidation of butyrate. The important discovery that fatty acid oxidation in animal tissue was chiefly localized in the mitochondrial particle gave needed support of the value of cytological localization of enzymes and their systems. Soon Green and Lynen and their collaborators tackled the problem of β-oxidation with modern enzyme technology and completely elucidated the problem. At present considerable interest is centered on the mechanism of action of the β-oxidation enzymes. The surprising discoveries of Wakil that fat synthesis involves a different and unique system quite apart from β-oxidation implies that synthesis and degradation are not in many cases interrelated by a mere reversibility of an enzyme reaction but are indeed complete and separate systems.

The cycle of events has made a complete turn for now the realm of research in this area is not that of new enzyme systems but of the clarification of problems more closely related to physiology: control of rate of synthesis and rate of breakdown; what makes a tissue choose between one or another system for its degradation sequence; what in a tissue's genetic and somatic heritage contributes to the factors that results in a seed tissue such as the pea with 1–2% of total fat whereas the peanut can contain as much as 60% of its dry weight as neutral lipid; what factors influence the ratio of unsaturated to saturated fatty acids;

what is the true role of lipoxidase and of lipase? There is a common denominator in the comparative biochemistry of fatty acid oxidation; namely β-oxidation. Superimposed on this are the variants that have developed in different tissues in response to their own needs. In some cases the life of the cell depends on the development and amplification of these variants but in other cases these play only a minor role. This chapter has attempted to point out these variants and indicate their relative importance.*

References

1. G. S. Parks and H. M. Huffman, "The Free Energies of Some Organic Compounds," p. 136. Chemical Catalog, New York, 1932.
2. H. Beinert, D. E. Green, P. Hele, H. Hift, R. W. Von Korff, and C. V. Ramakrishnan, *J. Biol. Chem.* **203,** 35–45 (1953); W. P. Jencks and F. Lipmann, *ibid.* **225,** 207–223 (1957).
3. A. Millerd and J. Bonner, *Arch. Biochem. Biophys.* **49,** 343–355 (1954).
4. P. K. Stumpf and G. A. Barber, *J. Biol. Chem.* **227,** 407–417 (1957).
5. P. Berg, *J. Biol. Chem.* **222,** 991–1013 (1956).
6. M. A. Eisenberg, *Biochim. et Biophys. Acta* **16,** 58–65 (1955).
7. A. Kornberg and W. E. Pricer, Jr., *J. Biol. Chem.* **204,** 329–343 (1953).
8. A. W. D. Avison, *J. Chem. Soc.* pp. 732–738 (1955).
9. W. P. Jencks, *Biochim. et Biophys. Acta* **24,** 227–228 (1957).
10. E. R. Stadtman, *J. Biol. Chem.* **203,** 501–512 (1953).
11. J. R. Stern, M. J. Coon, A. del Campillo, and M. C. Schneider, *J. Biol. Chem.* **221,** 15–31 (1956).
12. E. R. Stadtman, *J. Biol. Chem.* **196,** 527–534, 535–546 (1952).
13. T. A. M. Rose, M. Grunberg-Manago, S. R. Korey, and S. Ochoa, *J. Biol. Chem.* **211,** 737–756 (1954).
14. F. Lipmann, *J. Biol. Chem.* **155,** 55–70 (1944).
15. G. Kalnitsky and C. H. Werkman, *Arch. Biochem.* **2,** 113–124 (1943).
16. B. L. Horecker, E. C. Heath, J. Hurwitz, and A. Ginsburg, *Federation Proc.* **16,** 198 (1957).
17. H. J. Koepsell and M. J. Johnson, *J. Biol. Chem.* **145,** 379–386 (1942).
18. J. Gergely, *in* "Methods in Enzymology" (S. Colowick and N. O. Kaplan, eds.), Vol. I, pp. 602–607. Academic Press, New York, 1955.
19. J. R. Stern, M. J. Coon, and A. del Campillo, *Nature* **171,** 28–30 (1953).
20. J. R. Stern, A. del Campillo, and A. L. Lehninger, *J. Am. Chem. Soc.* **77,** 1073–1074 (1955).
21. W. P. McCann, *J. Biol. Chem.* **226,** 15–22 (1957).
22. W. Seubert, G. Greull, and F. Lynen, *Angew. Chem.* **69,** 359–361 (1957).
23. H. S. Moyed and F. Lipmann, *J. Bacteriol.* **73,** 117–121 (1957).
24. D. E. Green, S. Mii, H. R. Mahler, and R. M. Bock, *J. Biol. Chem.* **206,** 1–12 (1954).
25. F. L. Crane, S. Mii, J. G. Hauge, D. E. Green, and H. Beinert, *J. Biol. Chem.* **218,** 701–710 (1956).

* *Note added in proof:* Recent developments in this field have been discussed by Stumpf (*80*).

26. J. G. Hauge, F. L. Crane, and H. Beinert, *J. Biol. Chem.* **219,** 727–733 (1956).
27. H. Beinert and E. Page, *J. Biol. Chem.* **225,** 479–497 (1957).
28. H. Beinert, personal communication (1957).
29. J. G. Hauge, *J. Am. Chem. Soc.* **78,** 5266–5272 (1956).
30. H. M. Kalckar, *Chem. Revs.* **28,** 71–178 (1941).
31. R. G. Bartsch, Reactions of unsaturated fatty acids in *Clostridium kluyveri* extracts. Ph.D. Dissertation, University of California, Berkeley, California, 1956.
32. T. P. Singer, E. B. Kearny, and P. Bernath, *J. Biol. Chem.* **223,** 599–613 (1956).
33. T. P. Singer, V. Massey, and E. B. Kearny, *Arch. Biochem. Biophys.* **69,** 405–421 (1957).
34. T. P. Singer, E. B. Kearny, and V. Massey, *Advances in Enzymol.* **18,** 65–111 (1957).
35. V. Massey and T. P. Singer, *J. Biol. Chem.* **228,** 263–274 (1957).
36. S. Englard and S. P. Colowick, *J. Biol. Chem.* **221,** 1019–1035 (1956).
37. J. R. Stern, A. del Campillo, and I. Raw, *J. Biol. Chem.* **218,** 971–983 (1956).
38. J. R. Stern and A. del Campillo, *J. Biol. Chem.* **218,** 985–1002 (1956).
39. S. J. Wakil, *Biochim. et Biophys. Acta* **19,** 497–504 (1956).
40. T. C. Farrar, H. S. Gutowsky, R. A. Alberty, and W. G. Miller, *J. Am. Chem. Soc.* **79,** 3978–3980 (1957).
41. R. A. Alberty, W. G. Miller, and H. F. Fischer, *J. Am. Chem. Soc.* **79,** 3973–3977 (1957).
42. J. F. Speyer and S. R. Dickman, *J. Biol. Chem.* **220,** 193–208 (1956).
43. S. Englard and S. P. Colowick, *J. Biol. Chem.* **226,** 1047–1058 (1957).
44. J. R. Stern, *Biochim. et Biophys. Acta* **26,** 448–449 (1957).
45. A. L. Lehninger and G. D. Greville, *Biochim. et Biophys. Acta* **12,** 188–202 (1953).
46. J. R. Stern, A. del Campillo, and A. L. Lehninger, *J. Am. Chem. Soc.* **77,** 1073–1074 (1955).
47. S. J. Wakil, *Biochim. et Biophys. Acta* **18,** 314–315 (1955).
48. D. S. Goldman, *J. Biol. Chem.* **208,** 345–357 (1954); F. Lynen, *Federation Proc.* **12,** 683–691 (1953); J. R. Stern, M. J. Coon, and A. del Campillo, *J. Biol. Chem.* **221,** 1–14 (1956).
49. A. M. Siddiqi and A. L. Tappel, *J. Am. Oil. Chemists' Soc.* **34,** 529–533 (1957).
50. R. T. Holman and S. Bergstrom, *in* "The Enzymes: Chemistry and Mechanism of Action" (J. B. Sumner and K. Myrback, eds.), Vol. II, Part 1, pp. 559–580. Academic Press, New York, 1951.
51. A. M. Siddiqi and A. L. Tappel, *Plant Physiol.* **31,** 320–321 (1956).
52. W. Franke and H. Frehse, *in* "Handbuch der Pflanzenphysiologie" (W. Ruhland, ed.), Vol. VII, pp. 137–200. Springer, Berlin, 1957.
53. P. K. Stumpf, *J. Biol. Chem.* **223,** 643–649 (1956).
54. R. M. Burton and E. R. Stadtman, *J. Biol. Chem.* **202,** 873–889 (1953).
55. H. A. Barker and F. Lipmann, *Arch. Biochem.* **4,** 361 (1944); H. T. Johns, *Biochem. J.* **42,** 22 (1948); E. A. Delwiche, E. F. Phares, and S. F. Carson, *Federation Proc.* **13,** 198 (1954); H. R. Whiteley, *Proc. Natl. Acad. Sci. U. S.* **39,** 779–785 (1953); H. Larsen, *J. Biol. Chem.* **193,** 167 (1951).
55a. M. Flavin and S. Ochoa, *J. Biol. Chem.* **229,** 965–980 (1957); M. Flavin, H. Castro-Mendoza, and S. Ochoa, *J. Biol. Chem.* **229,** 981–996 (1957); W. S.

Beck, M. Flavin, and S. Ochoa, *ibid.* pp. 997–1010 (1957); J. Katz and I. L. Chaikoff, *J. Am. Chem. Soc.* **77,** 2659 (1955).
56. J. Giovanelli and P. K. Stumpf, *J. Am. Chem. Soc.* **79,** 2652–2653 (1957).
56a. G. Rendina and M. J. Coon, *J. Biol. Chem.* **225,** 523 (1957).
56b. P. R. Vagelos, *Federation Proc.* **18,** 342 (1959); P. R. Vagelos, J. M. Earl, and E. R. Stadtman, *J. Biol. Chem.* **234,** 490 (1959).
57. H. A. Barker and S. M. Taha, *J. Bacteriol.* **43,** 347–363 (1942).
58. B. T. Bornstein and H. A. Barker, J. Bacteriol. **55,** 223–230 (1948).
59. B. T. Bornstein and H. A. Barker, *J. Biol. Chem.* **172,** 659–669 (1948).
60. D. Rittenberg and K. Bloch, *J. Biol. Chem.* **160,** 417–424 (1945).
61. E. R. Stadtman and H. A. Barker, *J. Biol. Chem.* **180,** 1095–1115 (1949).
62. F. Lynen, E. Reichert, and L. Rueff, *Annal.* **574,** 1–32 (1951).
63. E. R. Stadtman, *Record Chem. Progr.* **15,** 1–17 (1954).
64. C. W. Shuster and I. C. Gunsalus, *Federation Proc.* **17,** 310 (1958).
64a. G. N. Cohen, *Ann. Rev. Microbiol.* **5,** 71–100 (1951).
65. H. A. Barker, *Arch. Mikrobiol.* **7,** 420–438 (1936).
66. T. C. Stadtman and H. A. Barker, *J. Bacteriol.* **61,** 67–80 (1951).
67. H. A. Barker, "Bacterial Fermentations." Wiley, New York, 1956.
68. J. H. Silliker and S. C. Rittenberg, *J. Bacteriol.* **61,** 653–659 (1951).
69. I. Iida, *Kekkaku* **28,** 229 (1953).
70. D. Ivler, J. B. Wolfe, and S. C. Rittenberg, *J. Bacteriol.* **70,** 99–103 (1955).
71. D. Murray and E. A. Dawes, *Biochem. J.* **63,** 20P (1956).
72. D. M. Webley, R. B. Duff, and V. C. Farmer, *Nature* **179,** 1130–1131 (1957).
73. S. Dagley, *Nature* **177,** 1131–1132 (1956).
74. H. Thaler and G. Geist, *Biochem. Z.* **302,** 369–383 (1939).
75. R. L. Girolami and S. G. Knight, *Appl. Microbiol.* **3,** 264–267 (1955).
76. H. Thaler and I. Stahlin, *Biochem. Z.* **320,** 84–86, 87–89 (1949).
77. R. J. W. Byrde, J. F. Harris, and D. Woodcock, *Biochem. J.* **64,** 154–160 (1956).
78. P. K. Stumpf and G. A. Barber, *Plant Physiol.* **31,** 304–308 (1956).
79. F. Lynen, *Ann. Rev. Biochem.* **24,** 653–688 (1955); E. P. Kennedy, *ibid.* **26,** 119–148 (1957); P. K. Stumpf and C. Bradbeer, *Ann. Rev. Plant Physiol.* **10,** 197–222 (1959).
80. P. K. Stumpf, Lipide Metabolism, *Ann. Rev. Biochem.* **29** (1960).

CHAPTER 4

Phosphoric Acid Anhydrides and Other Energy-Rich Compounds

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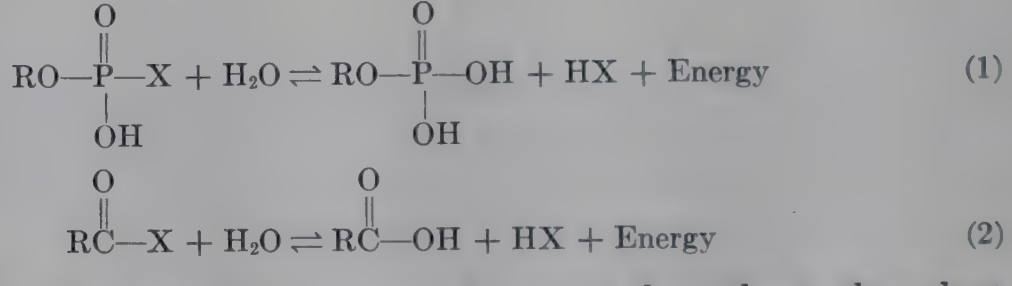
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I. Introduction

A. Energy Storage and Utilization in Living Systems

The terms "life" and "living systems" are difficult to define with any degree of precision (*1, 2*); for our purposes, it is sufficient to consider

living systems as those in which the replicative process is dependent upon energy derived from the breakdown of metabolites. In addition, most living systems require energy for maintenance during nonreplicative phases. Specifically, energy is utilized for chemical purposes such as the synthesis of metabolites or structural materials, or it may be used for mechanical work, as represented by the transport of materials or the movement of structural proteins. All types of energy-requiring reactions ultimately depend upon a rather small and highly specialized group of "energy-rich" chemical compounds to supply the driving force. From a structural viewpoint, these compounds are limited to anhydride, thioester, and amidine derivatives of phosphoric or carboxylic acids, and the energy is released during the hydrolytic scission of the bond joining phosphoric acid (Reaction 1) or the carboxylic acid (Reaction 2) to its derivative.



$$RO{-}P(=O)(OH){-}X + H_2O \rightleftharpoons RO{-}P(=O)(OH){-}OH + HX + \text{Energy} \quad (1)$$

$$RC(=O){-}X + H_2O \rightleftharpoons RC(=O){-}OH + HX + \text{Energy} \quad (2)$$

Certain of the "energy-rich" compounds occur throughout the plant and animal kingdoms, whereas others are limited in their distribution.

The relatively efficient transfer of energy from one "energy-rich" compound to another has made it obligatory to analyze a living system for the *entire complement* of these compounds in order to assess the total "energy potential" of the system. An accurate determination of the energy potential is an obvious prerequisite to the understanding of such paramount problems as growth, replication, maintenance, and aging in the normal system, or disease in the abnormal system. For example, it is well known that fatigue in muscle tissue is caused by the temporary depletion of adenosine triphosphate (ATP)* and phosphocreatine (3). Similarly, in the human erythrocyte, the viability of

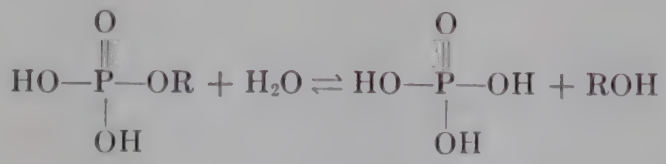
* The following abbreviations will be used: ATP, ADP, and AMP, adenosine tri-, di-, and monophosphate; ITP etc., GTP etc., CTP etc., UTP etc., the corresponding tri-, di-, and monophosphates of inosine, guanosine, cytidine, and uridine; P_i, inorganic orthophosphate; PP, inorganic pyrophosphate; DPN, DPNH, TPN, TPNH, oxidized and reduced diphosphopyridine nucleotide and triphosphopyridine nucleotide; PEP, phosphoenolpyruvate; CoA, coenzyme A; ΔF, ΔH, and ΔS, changes in free energy, enthalpy (heat content), and entropy; ΔF°, change in free energy under standard conditions (see text for further explanation); K, equilibrium constant; T, absolute temperature; pK, log of the reciprocal of a dissociation constant.

the cell during *in vitro* storage is directly correlated to the level of endogenous ATP and other phosphate compounds (*4*).

B. Phosphorylated Compounds and Their Role in Energy Storage

Throughout the 1800's and early 1900's, the study of living systems was confined, almost of necessity, to an analysis of constituent substances. Carbon, hydrogen, oxygen, and nitrogen were identified as key elements in biological substances, and shortly thereafter the widespread occurrence of phosphorus was noted. In 1847 Liebig (*5*) isolated from mammalian muscle a crystalline material which was subsequently identified as inosinic acid (inosine-5-phosphate). However, many years intervened between this discovery and Miescher's monumental studies in 1869 on the isolation and partial characterization of nucleic acid (*6*). The 1920's and 1930's witnessed the isolation of other naturally occurring phosphate esters [e.g., ATP (1929), DPN (1936), diphosphothiamine (1937), flavin adenine dinucleotide (1938), and various hexose phosphates].

Work from Meyerhof's laboratory in the 1930's drew attention to the synthesis of ATP during glycolysis, and also to the requirement for this compound and phosphocreatine in the process of muscle contraction. Meyerhof first measured, by calorimetric techniques, the ΔH of hydrolysis for a number of biologically important phosphate esters and anhydrides (Eq. 3):



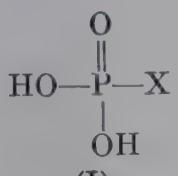
(3)

From these measurements, and from ΔF determinations, Meyerhof recognized that certain phosphate esters or anhydrides possessed "energy-rich" bonds, whose hydrolysis was characterized by ΔF and ΔH values of -8 to -12 kcal./mole. We shall see subsequently, however, that these values were uniformly high by about 4 kcal./mole.

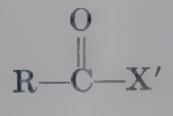
The concept of "energy-rich" phosphate bonds was developed more fully in the reviews of Lipmann (*7*) and Kalckar (*8*). These authors drew attention to ADP, phosphocreatine, phosphoarginine, acetyl phosphate, phosphoenolpyruvate, and glyceryl phosphate as additional members of the "energy-rich" category. By contrast, most of the common phosphorylated compounds, such as glucose-6-phosphate, inositol phosphate, or choline phosphate, were shown to contain "energy-poor" phosphate linkages, which yielded only 2–4 kcal./mole upon hydrolysis. More recently, other "energy-rich" compounds not involving a phos-

phate group as either partner of the susceptible bond (e.g., acetyl-CoA) have been discovered.

The present chapter surveys the various "energy-rich" compounds, which represent potential or actual energy reserves in living systems. These compounds fall into two general structural categories, as represented in Fig. 1.



(I)

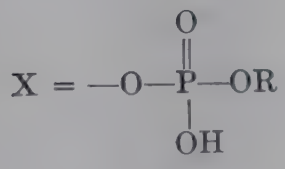


(II)

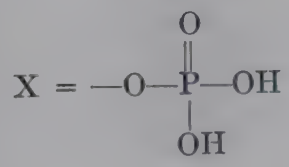
FIG. 1. "Energy-rich" structures. I. Derivatives of phosphoric acid; II. Derivatives of carboxylic acid.

The Type I compounds are derivatives of phosphoric acid and include:

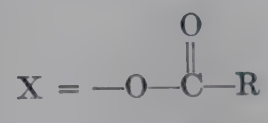
nucleoside polyphosphates*



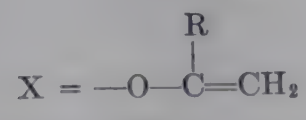
inorganic polyphosphates



acyl phosphates



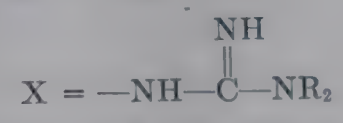
enol phosphates



thiol phosphates



amidine phosphates

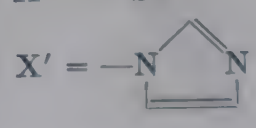


The Type II compounds are derivatives of carboxylic acids and include:

acyl thioesters



acyl imidazoles



* We have omitted from the discussion the nucleotide coenzymes such as DPN, TPN, flavin adenine dinucleotide, coenzyme A, and uridine diphosphate glucose. These compounds contain an "energy-rich" pyrophosphate linkage which is not available for energy-requiring reactions. Ammonium and sulfonium compounds such as betaine, the thetins, and S-adenosylmethionine (all of which function as methyl group donors) might also be considered "energy-rich" compounds but are not anhydrides and, hence, have been omitted from this discussion.

II. Chemical and Thermodynamic Aspects of "Energy-Rich" Bonds

A. Chemical Basis for "Energy-Rich" Phosphate Bonds

Fundamental concepts of organic and physical chemistry have been used to provide explanations for the "energy-rich" nature of certain phosphate compounds. Lipmann (*7*) commented upon the fact that three of the most prominent examples of this class of compounds (ATP, acetyl phosphate, and PEP) were *anhydrides* of phosphoric acid and a second acid (i.e., phosphoric acid, a carboxylic acid, and an enol, in the above three cases). Contrary to this generalization, however, was the obvious fact that the copartner of phosphoric acid in phosphocreatine contains a very basic nitrogen group.

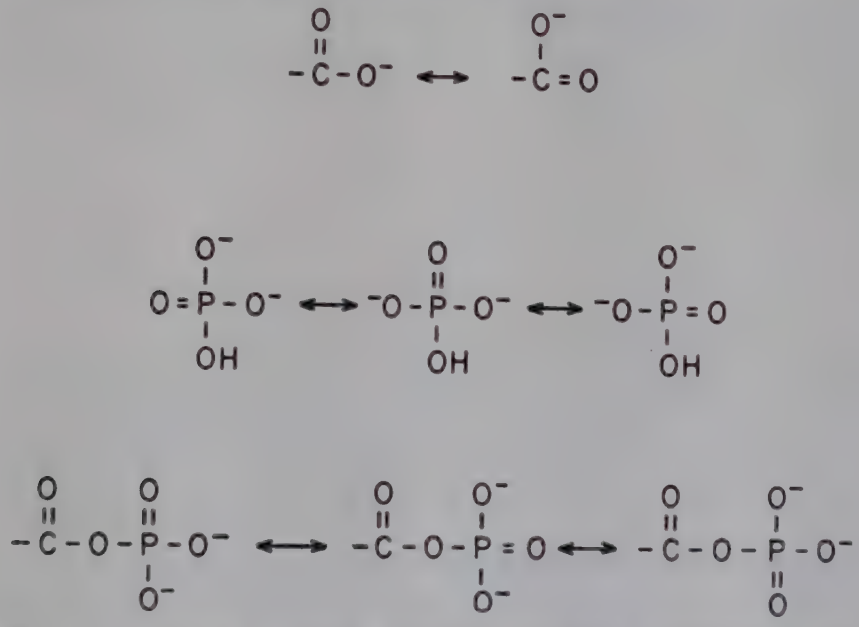


Fig. 2. Resonance forms of carboxylate and phosphate ions.

Although this consideration failed to provide a uniform explanation for the nature of the "energy-rich" linkages, Lipmann's approach in terms of organic and physical chemistry set the pattern for subsequent examination of this problem. Kalckar (*8, 9, 10*) drew attention to the difference in resonance stabilization between the "energy-rich" compound and its hydrolysis products. Using carboxyl phosphate as an example, Kalckar noticed that certain of the principal resonance forms of the carboxylate ion and the phosphate ion, as shown in Fig. 2, were blocked by "opposing resonance" when the carboxyl and the phosphate groups were joined together; he implied that the blocking of such mesomeric forms was a characteristic of *all* "energy-rich" compounds. Since the thermodynamic stability of a substance is increased by virtue of its ability to assume multiple mesomeric forms, it follows that the

carboxylate ion and the phosphate ion are both stabilized by resonance to a greater degree than the carboxyl phosphate and will have, therefore, a lower energy than the latter compound. Thus, hydrolysis of carboxyl phosphate would yield more energy than hydrolysis of an alcohol phosphate, such as glucose-6-phosphate, since the alcohol produced in the latter reaction does not have multiple resonance forms.

The formulation of Kalckar can be visualized by the energy diagram shown in Fig. 3. ΔF_1 is the inherent free energy of hydrolysis associated

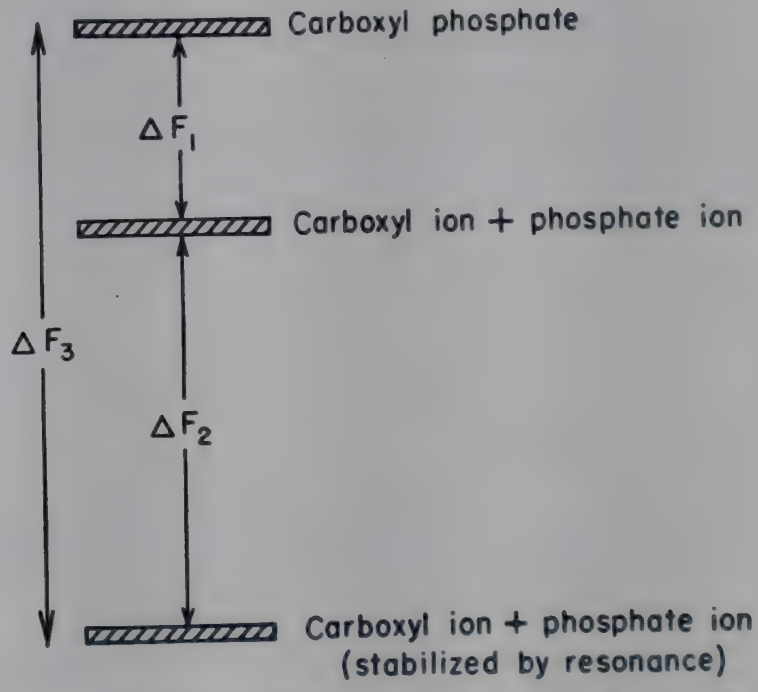
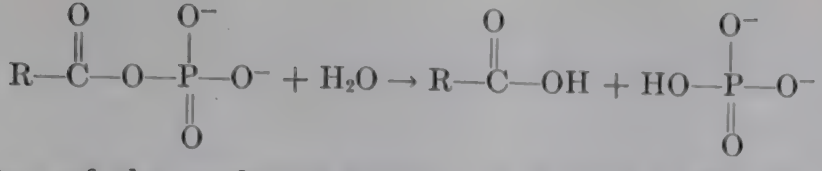


FIG. 3. Effect of resonance stability on the ΔF of hydrolysis.

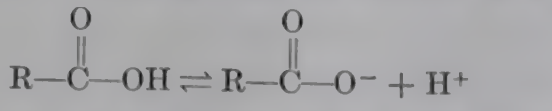
with the scission of the phosphate bond; presumably, this has a constant value for all compounds having an O—P bond, but a different value for those having an N—P bond. ΔF_2 is the extra increment of energy provided by the relative stability (as measured *qualitatively* by the number of possible resonance forms) of the products as compared with the reactants. This in an appreciable factor only in the case of the "energy-rich" compounds. ΔF_3 is the total, or observed, free energy of hydrolysis.

Oesper's (*11*) considerations were also based upon resonance stabilization of the hydrolysis products, but, in addition, he stressed the important fact that the total free energy of hydrolysis of a compound was apportioned between the inherent energy of hydrolysis and the energy of ionization if the reactants and products had markedly different pK values. We shall see subsequently how a proper evaluation of this latter term caused the ΔF value for ATP hydrolysis to be revised downward (cf. Section II,D). Using the previously cited example, the hydrolysis of carboxyl phosphate is shown in Eq. 4.



(4)

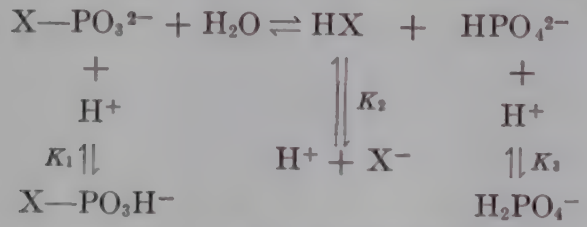
The ionization of the carboxyl group, as shown in Eq. 5, would con-



(5)

tribute a further term of −3.2 kcal./mole* to the total ΔF of hydrolysis if the dissociation constant, K_1, of the carboxyl group is assumed to be 2×10^{-5} and the hydrolysis is carried out at pH 7.

The theoretical approach was further expanded by Hill and Morales (*13*). These writers utilized the concepts developed previously by Kalckar and Oesper, but added refinements in the form of a semiquantitative interpretation of the contribution of various factors to the "energy-rich" nature of these compounds. A typical hydrolysis reaction, accompanied by the principal ionization effects, is depicted in Eq. 6:



(6)

ΔF° is the free energy change for the *primary reaction* as written across the top line; ΔF°_1, ΔF°_2, and ΔF°_3 are the free energy changes for the corresponding ionization reactions indicated by K_1, K_2, and K_3. $(\Delta F^\circ)_a$ is the free energy change for the *actual* equilibrium mixture of the various ionized forms of reactants and products. The contribution of the dissociation reactions was treated by Hill and Morales as corrections to the primary reaction:

$$(\Delta F^\circ)_a = \Delta F^\circ + \Delta F^\circ_2 + \Delta F^\circ_3 - \Delta F^\circ_1 \tag{7}$$

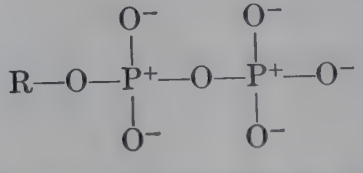
The hydrolysis of the phosphate bond in AMP was taken as the prototype for an "energy-poor" bond; the experimental value for this reaction is ca. −2 kcal./mole (*7*, *14*). For typical "energy-rich" compounds, such as acetyl phosphate, 1,3-diphosphoglycerate, phosphoenolpyruvate, phosphocreatine,† phosphoarginine, adenosine diphosphate,

* Oesper used the expression $\Delta F = RT \ln [(H^+)/K]$ for this calculation; later Hill and Morales (*12*) suggested the more rigorous function

$$\Delta F = -RT \ln \left[1 + \frac{K}{(H^+)} \right].$$

† *N*-Phosphates can be treated similarly to the *O*-phosphate compounds, since the larger stability (by approx. 28 kcal./mole) of the *O*-phosphate bond, as compared to the *N*-phosphate bond, is nearly compensated by the greater stability of the O—H bond in the product (approx. 26.5 kcal./mole).

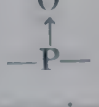
and adenosine triphosphate, the free energy of hydrolysis was taken as ca. —10 kcal./mole, although this value is now known to be high. For each of the above compounds, Hill and Morales assessed the various factors that contribute to the enhanced free energy of hydrolysis over AMP. The largest contribution in all cases is due to the relative resonance stabilization of the products, but no quantitative assessment can be made of this factor. The pK values for the dissociation reaction in Eq. 6 were converted into values for ΔF°_1, ΔF°_2, and ΔF°_3. In addition to resonance stabilization and ionization, these authors suggested an additional contributing factor, electrostatic repulsion, which is certainly applicable to ATP and ADP, and possibly to phosphoenolpyruvate. This repulsion is caused by the positive centers‡ on adjacent phosphate atoms, *viz.*:



and is estimated from a theoretical model to be 5–6 kcal./mole for ATP and 3–4 kcal./mole for ADP and phosphoenolpyruvate.

Finally, the conclusions drawn by Hill and Morales may be recapitulated briefly: the ΔF of hydrolysis is not a reflection of a particular, localized *bond* energy, but is, rather, the difference in free energies between the products and reactants; and important contributions to the net difference in free energy may be provided by resonance stabilization, ionization effects, and electrostatic repulsions. Thus, the term§ "energy-rich," as customarily applied to a certain class of phosphate compounds, is erroneous. On the other hand, the term is admittedly convenient and vivid, and it will continue to be used in this chapter with the foregoing reservations implicitly understood. Accordingly, we shall define an "energy-rich" compound as one that undergoes hydrolysis at a particular bond with a ΔF° value greater than —5 kcal./mole.

‡ The P=O double bond is more correctly written (*15*) as a "semi-polar" double bond:



§ We have preferred to use the expression "energy-rich" rather than the equally widespread term "high energy" as applied to phosphate compounds; the latter term is often denoted by the symbol ~P. Gillespie *et al.* (*15a*) have also cautioned against the improper use, in a thermodynamic sense, of the term "high energy" bond.

B. Thermodynamic Concepts and Terminology*

Any reversible chemical reaction may be fully defined, in a thermodynamic sense, by three parameters: (*a*) the change in free energy, ΔF, which is the maximum amount of energy available as useful work; (*b*) the change in heat content, or enthalpy, ΔH, the total energy; and (*c*) the change in entropy, ΔS, or the unavailable energy. At a given temperature, T, these fundamental quantities are related by the well-known equation emerging from the Second Law of Thermodynamics:

$$\Delta F = \Delta H - T\,\Delta S \tag{8}$$

ΔH may be measured directly for a reaction by calorimetric procedures, while ΔF is usually calculated from other thermodynamic parameters as discussed in the succeeding section. The entropy term is less accessible, and is usually calculated from Eq. 8 when the other terms are known. If the entropy term is small, ΔF may be taken as equal to ΔH. The customary units† are kcal./mole for ΔF and ΔH, and kcal./mole/degree for ΔS. For a comprehensive summary of thermodynamic values for many biological reactions, including the hydrolysis of "energy-rich" compounds, the reader may consult several comprehensive reviews (*7, 8, 11, 13, 21, 23–28*) and Vol. II, Chapter 1 in this treatise.

It is convenient to define ΔF as ΔF° under a set of arbitrarily chosen standard conditions.‡ For all solutes the "standard" concentration§ is 1.0 M. ΔF° is related to the equilibrium constant, K, by the equation:

$$\Delta F^\circ = -RT \ln K \tag{9}$$

R has the value of 1.98 kcal./mole, $T = 273 + t^\circ\text{C}$, and $\ln K = 2.3 \log K$. For other than standard conditions, ΔF may be expressed in terms of ΔF° and the actual concentration of reactants and products by Eq. 10.

$$\Delta F = \Delta F^\circ + RT \ln \frac{(\text{products})}{(\text{reactants})} \tag{10}$$

* For a more detailed treatment of thermodynamics, the reader may consult any of the standard texts (*16*) on this subject; applications to biochemical reactions are discussed in reviews (*7, 17–21*).

† When ΔF is negative, the reaction is *exergonic* (*22*) and tends to proceed spontaneously; when ΔF is positive the reaction is *endergonic*.

‡ ΔH might also be defined as ΔH° under standard conditions, but this is usually not necessary since ΔH is nearly independent of concentrations.

§ If the solvent is water, as it is in nearly all biochemical reactions, the concentration of water is 55.5 M. In equations such as Eq. 3, where water is also a reactant or product, the amount of water generated or consumed during the reaction is negligible compared to that present as the solvent. Consequently, the term for water concentration is usually omitted from the expression for the equilibrium constant.

At equilibrium $\Delta F = 0$ and $\Delta F^\circ = -RT \ln K$, as before. ΔF° values have the desirable feature of uniformity and can be added or subtracted directly when equations involving the compounds are combined. ΔF values, on the other hand, differ with various concentrations and are, therefore, of limited value in making comparisons.

C. Methods for Determining ΔF and ΔH

1. *Calorimetric Measurement of ΔH of Hydrolysis*

By employing the standard calorimetric apparatus and technique (*29*, *30*), it has been possible to determine ΔH directly for a number of enzymatic reactions including the hydrolysis of some phosphorylated compounds (*31*, *32*). Measurements of ΔH were initially of considerable value in differentiating "energy-rich" and "energy-poor" phosphate compounds.

2. *Calculation of ΔF° from Thermal Data*

ΔF° for a reaction may be calculated from known or estimated data of free energy of formation for the reactants and products. For example, Lipmann's estimation (*7*) of the ΔF° value for the hydrolysis of phosphoenolpyruvate (Reaction 11) made use of this method.

$$\text{Phosphoenolpyruvate} + H_2O \rightarrow \text{Pyruvate} + P_i \qquad (11)$$

This reaction may be considered as the sum of three other reactions:

$$\text{Phosphoenolpyruvate} + H_2O \rightleftharpoons \text{Phosphoglycerate} \qquad (12)$$

$$\text{Phosphoglycerate} + H_2O \rightarrow \text{Glycerate} + P_i \qquad (13)$$

$$\text{Glycerate} \rightarrow \text{Pyruvate} + H_2O \qquad (14)$$

Reactions such as Eq. 12, involving the hydration of a double bond, usually have a ΔF° value of less than -1 kcal./mole and may be neglected. The ΔF° for Reaction 13 may be estimated at -3 kcal./mole, which is an average value for "energy-poor" phosphate compounds (*7*, *24*, *27*). The dehydration Reaction 14 is undoubtedly more complicated in a mechanistic sense than Reaction 12* and it might well be expected, *a priori*, to have a considerably larger ΔF° value. Using the heats of *combustion* for the components of Reaction 14, Lipmann (*7*) calculated that ΔF° = approx. -13 kcal./mole; Kaplan (*23*) arrived at a value of -14 kcal./mole from the free energies of *formation*. The average of these two values, -13.5 kcal./mole, when added to

* Reaction 12 is similar to the reaction:

$$\text{Fumarate} + H_2O \rightleftharpoons \text{Malate}$$

which has a ΔF° value of -0.88 kcal./mole (*27*).

ΔF° for Reaction 13 of -3 kcal./mole and $\Delta F^\circ = 0$ for Reaction 12 give an over-all figure of $\Delta F^\circ = -16.5$ kcal./mole for Reaction 11. Other methods have yielded a lower value for the ΔF° of hydrolysis of phosphoenolpyruvate (cf. Table I).

3. *Calculation of ΔF° from Equilibrium Data*

Many phosphate compounds can be brought into equilibrium with one another by means of transphosphorylating enzymes. This type of linked reaction can be used to determine an unknown ΔF° of hydrolysis from a known value as, for example, in Bücher's (*33*) study of the reaction:

$$\text{1,3-Diphosphoglycerate} + \text{ADP} \rightleftharpoons \text{3-Phosphoglycerate} + \text{ATP} \qquad (15)$$

The equilibrium constant for this reaction was found to be 3×10^3. At 25°, ΔF° for the reaction would be -4.3 kcal./mole according to Eq. 9. Since the reaction ATP $\rightarrow$ ADP has a ΔF° value of -7.0 kcal./mole (cf. Section II,D), ΔF° of hydrolysis for glyceryl phosphate is -11.3 kcal./mole.

4. *Calculation of ΔF° from Hydrolysis Data*

From Eq. 9 the ΔF° for hydrolysis can be ascertained directly if the reaction is measurable under equilibrium conditions. Obviously, for "energy-rich" phosphate esters the hydrolytic reaction proceeds too far to permit an equilibrium to be measured: for example, if $\Delta F = -10$ kcal./mole, K is 2×10^7. For "energy-poor" compounds, however, K is usually not larger than 10^2. Lipmann (*7*) has used Kay's data (*34*) for the hydrolysis of glycerophosphate by intestinal phosphatase to calculate a value of $\Delta F^\circ = -2.28$ kcal./mole. For this reaction Colowick (*35*) made the interesting observation that if all the reactants, including water, were in their true standard state, i.e., at 1 M, ΔF° would be $+0.2$ kcal./mole. In the aqueous medium where the hydrolysis is carried out, the concentration of water is 55.5 M. The change in concentration of water from 1 M to 55.5 M contributes -2.5 kcal./mole, thereby giving the commonly used ΔF° value of -2.3 kcal./mole.

D. The Primary Standard: ΔF° for the Hydrolysis of ATP

From the preceding discussion it is evident that there are relatively few independent measurements for establishing the ΔF° values of the "energy-rich" compounds. Most of the methods require an accurate value for a given ΔF° in order to determine the value in question. It is evident that the ΔF° value for ATP is the most widely applicable primary standard.

From 1934, when the earliest calorimetric measurements were made (*31*, *32*), until 1956, the ΔH value for ATP was taken as −12 kcal./mole, and the $\Delta F°$ value was assumed to be approximately the same. Recent measurements, however, have resulted in a decrease in both the $\Delta F°$ and ΔH values of ATP. Podolsky and Sturtevant (*36*) measured calorimetrically the ΔH value for ATP hydrolysis catalyzed by purified myosin ATPase. The measured value of ΔH, ΔH_{obs}, at pH 8.0 was −15.9 kcal./mole. This value is equal to the sum of ΔH for the hydrolysis of the substrate and ΔH_b, the heat of neutralization of the H^+ (produced in the primary reaction) by the buffering medium. Thus,

$$\Delta H_{obs} = \Delta H + \Delta H_b \tag{16}$$

For the primary hydrolytic reaction we may write:

$$ATP^{4-} + H_2O \rightarrow ADP^{3-} + P_i^{2-} + H^+ \tag{17}$$

From the known dissociation constants (*37*) for ATP, ADP, and P_i, it can be shown that at pH 8.0, 0.97 mole of H^+ is liberated per mole of ATP hydrolyzed. ΔH_b is −10.6 kcal./mole for the neutralization of that amount of H^+, and, therefore, ΔH for the primary reaction is only −5.3 kcal./mole. Later, Podolsky and Morales (*38*) extended these calorimetric measurements and obtained a value of ΔH equal to −4.7 kcal./mole at pH 8.0. Values for ΔH near −5 kcal./mole are more consistent with those for inorganic pyrophosphate (*39*) and trimetaphosphate (*40*).

The $\Delta F°$ value for ATP hydrolysis has also been revised downward. Levintow and Meister (*41*) measured the equilibrium of the reaction:

$$ATP + NH_3 + \text{Glutamate} \rightleftharpoons \text{Glutamine} + ADP + P_i \tag{18}$$

and obtained an equilibrium constant, K, equal to 1.2×10^3 for this reaction at pH 7.0 and 37°. From this value of K, it can be calculated that the $\Delta F°$ of hydrolysis for ATP is larger by 4.3 kcal./mole than the $\Delta F°$ for glutamine hydrolysis catalyzed by glutaminase (Eq. 18a). The absolute value of $\Delta F°$ for ATP can be obtained

$$\text{Glutamine} + H_2O \rightleftharpoons \text{Glutamate} + NH_3 \tag{18a}$$

if the $\Delta F°$ for Reaction 18a is known. The above authors assumed that Borsook's value of −3.7 kcal./mole (*41a*) for the hydrolysis of an ordinary peptide bond was applicable to the amide hydrolysis in Reaction 18a. $\Delta F°$ for ATP was thus calculated to be −8.0 kcal./mole. Later, Morales *et al.* (*42*) corrected these calculations for the ionization of the various reacting species in Eqs. 17, 18, and 18a and obtained a figure of −7.0 kcal./mole for ATP hydrolysis.

A further complication of this problem relates to the binding of Mg^{2+}

by various reactants in the above equations. Thus, Burton (*43*) has shown by direct measurement of affinity constants that Mg^{2+} is more tightly bound to ATP than to ADP, with the result that the ΔF° value for ATP hydrolysis in the *absence* of Mg^{2+} (Eq. 17) will be more negative by 1.6 kcal./mole than the ΔF° value in the presence of excess Mg^{2+} (cf. Reaction 18b).

$$MgATP^{2-} + H_2O \rightarrow MgADP^- + HPO_4^{2-} + H^+ \qquad (18b)$$

Considering the effect of Mg^{2+} binding, and using new data for Reaction 18a in conjunction with Levintow and Meister's (*41*) original value for glutamine synthesis, Benzinger *et al.* (*43a–43c*) have concluded that the best value for ΔF° of ATP hydrolysis at pH 7.0 and 37° is —8.6 kcal./mole in the absence of Mg^{2+}, and —7.0 in the presence of excess Mg^{2+}.

A parallel approach to the problem of obtaining the ΔF° value for ATP hydrolysis was made by Robbins and Boyer (*44*) who used C^{14}-labeled substrates to measure the equilibrium of Reaction 19 in *both* directions.

$$ATP + Glucose \rightleftharpoons Glucose\ 6\text{-}phosphate + ADP \qquad (19)$$

At pH 7.0 and 30°, ΔF° was found to be —4.7 kcal./mole for this reaction. The ΔF° value for the hydrolysis of glucose-6-phosphate (Eq. 20) was calculated from earlier data of Meyerhof and Green (*44a*)

$$Glucose\text{-}6\text{-}phosphate + H_2O \rightleftharpoons Glucose + P_i \qquad (20)$$

to be —3.1 kcal./mole. Summation of Reactions 19 and 20 yields the reaction for ATP hydrolysis and a ΔF° value of —7.8 kcal./mole. The equilibrium constant of the hexokinase reaction (19) is also affected by Mg^{2+} but in a different manner than the glutaminase reaction (18), owing to the binding by the metal of an additional compound, glucose-6-phosphate. Thus, the above figure (—7.8 kcal./mole) was determined in the presence of a high concentration of Mg^{2+}, whereas a lower value (—7.6 kcal./mole) is found in the absence of Mg^{2+}.

Klotz (*20*) has discussed the hydrolysis of ATP under *physiological conditions* for which the above ΔF° must be corrected by a term involving the actual concentrations of the various reactants and products, i.e.,

$$\Delta F = \Delta F^\circ + RT \ln \frac{(ADP)(HPO_4)}{(ATP)} \qquad (21)$$

It is difficult, of course, to estimate the concentrations of either the reactants or the products under physiological conditions, but making the reasonable assumption that the concentrations of ADP and ATP are

approximately equal, and that the concentration of HPO_4 is approx. 10^{-2} M, the second term on the right side of Eq. 21 will be negative. In fact, at any concentration of HPO_4 below 1 M, ΔF will be more negative than $\Delta F°$.

The reduction in thermodynamic values for ATP hydrolysis has evoked the following apt comment by Morales *et al.* (see reference *42*, p. 476):

"Some time ago we suggested that substances like ATP, whose hydrolyses were thought to be highly exergonic, not be described as compounds containing a 'high-energy phosphate bond,' for this description violated established concepts of both 'bond' and 'energy'; possibly it also violates the established concept of 'high.'"

E. Summary of Thermodynamic Values for the Hydrolysis of "Energy-Rich" Compounds

It is apparent from the above discussion that the inherent value of the $\Delta F°$ for hydrolysis of ATP is affected by the following factors: (*a*) pH; (*b*) temperature; and (*c*) concentration of divalent metal ion (usually Mg^{2+} or Mn^{2+}). To this should be added the possible effect of the enzyme (if present at an appreciable concentration) in shifting the equilibrium of the reaction (*44b*, *44c*). Thus, the accuracy of any reported value of $\Delta F°$ for ATP is dependent upon the extent to which the above parameters were controlled in the original measurements. For the purpose of this review, we have chosen to use the most recently determined value (*43c*) of −7.0 kcal./mole for the $\Delta F°$ of ATP at pH 7.0, 25°, excess Mg^{2+}, and catalytic quantities of enzyme.

All of these conditions apply, as well, in coupled reactions where the $\Delta F°$ of another "energy-rich" compound is to be determined from the measured equilibrium constant of the reaction between the compound and ATP. Unfortunately, most of the existing equilibrium data for the coupled reactions have been obtained under diverse conditions of pH and temperature, while the effects of metal ion and enzyme concentration have usually not been considered. Approximate values (probably accurate to only ±1–2 kcal./mole) for the $\Delta F°$ of hydrolysis for other "energy-rich" compounds (summarized in Table I) were obtained by attempting to correct the data to a uniform set of conditions, i.e., pH 7.0 and 25°, and making the assumption that the original determinations were made in the presence of Mg^{2+}.

The above $\Delta F°$ values are used most frequently in *linked* reactions, e.g.,

$$\text{ATP} + \text{Creatine} \rightleftharpoons \text{ADP} + \text{Phosphocreatine} \qquad (22)$$

where the terms for water and HPO_4^{2-} in the individual hydrolytic reactions are cancelled out, and also the pK values for the two conjugate pairs of substances in the equation are nearly equal. Owing to the latter fact, the effect of pH upon the equilibrium of a coupled reaction is usually less pronounced than in the hydrolysis of either of the two

TABLE I
APPROXIMATE $\Delta F°$ VALUES FOR HYDROLYSIS OF "ENERGY-RICH" COMPOUNDS

Compounds	$-\Delta F°$ kcal./mole	References
Pyrophosphate	6.0	*40*
Acetyl coenzyme A	6.3	*25*
Adenosine diphosphate	6.5	*27a*
Adenosine triphosphate[a]	7.0	*43c*
Adenosine triphosphate[b]	8.6	—[c]
Acetyl phosphate	8.7	*24, 25*
S-Phosphoryl coenzyme A	9.0	*49, 49a*
Phosphocreatine	9.8	*46*
Glyceryl phosphate	11.3	*33*
Phosphoenol pyruvate	12.4	*45–45b*
Acetyl adenylate	13.0	*47*
Acetyl imidazole	13.3	*48*
Adenosine phosphosulfate	18.0	*44c*

[a] ATP $\rightarrow$ ADP + P_i.
[b] ATP $\rightarrow$ AMP + PP.
[c] Calculated from $\Delta F°$ values for hydrolysis of ATP, ADP, and PP.

energy-rich compounds. Finally, it must be borne in mind that the measured ΔF changes for any reaction can only be interpreted correctly by writing the reaction in full, and by taking cognizance of all the ionic species involved as well as the possible interactions between the various reactants and metal ions, enzyme, salts, etc.

III. "Energy-Rich" Compounds in Biological Systems

A. Derivatives of Phosphoric Acid

1. *Nucleoside Polyphosphates: Adenosine Triphosphate and Related Compounds*

a. Preparation and Identification. The nucleoside polyphosphates may be considered as mixed anhydrides of phosphoric acid and a substituted phosphoric acid. From the viewpoint of energy storage, the most important representative of this class of compounds is adenosine-

5′-triphosphate (ATP), followed to a lesser extent by adenosine-5′-diphosphate (ADP) and the recently discovered adenosine-5′-tetraphosphate. We shall also include in the discussion, although briefly, the polyphosphates of other nucleosides: guanosine, inosine, cytidine, and uridine. The structures of these compounds are shown in Fig. 4.

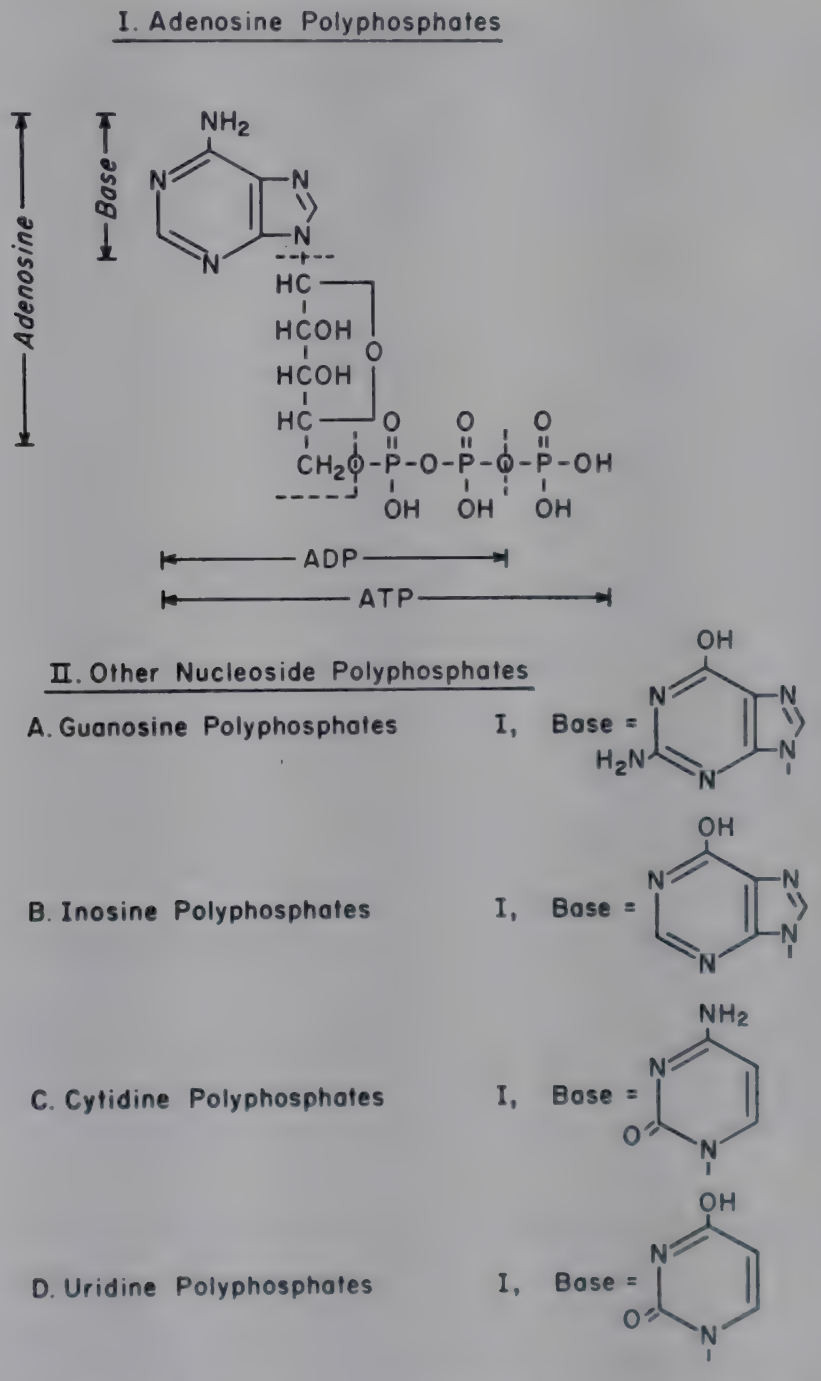


FIG. 4. Structures of nucleoside polyphosphates.

The nucleoside polyphosphates are found in a wide variety of cells and tissues and it is evident that the prototype, ATP, is the primary phosphorylating agent for most enzymatic reactions (50), and hence,

the primary energy source for many physiological processes. To a much lesser extent, the other nucleoside triphosphates (NTP) may act as phosphorylating agents for specific reactions, or they may transphosphorylate (*51–54b*) with ADP to yield ATP:

$$\text{NTP} + \text{ADP} \rightleftharpoons \text{NDP} + \text{ATP} \tag{23}$$

ATP was first isolated as the barium salt from muscle extracts in 1929 by Lohmann (*55*) and by Fiske and SubbaRow (*56*). The "energy-rich" nature of ATP was recognized by Meyerhof on the basis of calorimetric measurements (*31, 32*) of the ΔH of hydrolysis and calculations (*28*) of the ΔF° of hydrolysis. ATP has been isolated from muscle by a number of procedures (*57–60*), all employing fractionation with heavy metals and solvents. More recently, anion-exchange chromatography (*61*) using gradient elution (*62*) has been used with considerable success for both the isolation and the identification of ATP and other nucleoside polyphosphates. ADP is customarily prepared by the careful hydrolysis of ATP using an ATPase isolated from lobster muscle (*57, 63, 64*). Adenosine tetraphosphate has been isolated (*65, 66*) from horse muscle and shown to be a linear 5′-tetraphosphate of adenosine; as yet, no metabolic function has been ascribed to this compound.

The chemical synthesis of ADP and ATP has been achieved by Todd and his colleagues (*67, 68*) through the phosphorylation of the isopropylidine derivative of AMP with dibenzyl phosphorochloridate, followed by removal of the various protective groups. Improved methods, employing dehydrating agents such as the carbodiimides (*69, 69a*), were also utilized for the direct combination of phosphoric acid with AMP or ADP. The chemical synthesis of UDP and UTP by the carbodiimide method has been reported (*70*).

ATP, ADP, adenosine tetraphosphate, and other nucleoside di- and triphosphates may be separated from one another and quantitatively estimated by means of ion-exchange chromatography (*61, 62, 71*). This method is undoubtedly the most sensitive one available and is preferable for complex mixtures of these materials. Paper chromatography, likewise, has been used in many investigations (see, for example, Bock *et al.*, *72*) for the separation and identification of these nucleotides. Colorimetric methods, based upon the release of P_i after 7-minute hydrolysis at 100° in 1 *N* acid, or based upon solubility in barium-alcohol fractionation procedures, are relatively nonspecific, and have been largely replaced by the more sensitive chromatographic methods.

Enzymatic assays (*73–76*), based upon the following groups of reac-

tions (24a,b; 25a,b; and 26–28), have also been employed for the quantitative estimation of the adenosine polyphosphates:

$$\text{Glucose} + \text{ATP} \xrightarrow{\text{hexokinase}} \text{Glucose-6-phosphate} + \text{ADP} \quad (24a)$$

$$\text{Glucose-6-phosphate} + \text{TPN}^+ \xrightarrow{\text{G-6-P dehydrogenase}} \text{6-Phosphogluconate} + \text{TPNH} + \text{H}^+ \quad (24b)$$

$$\text{Luciferin} + \text{ATP} \rightarrow \text{Luciferyl-AMP} + \text{PP} \quad (25a)$$

$$\text{Luciferyl-AMP} \xrightarrow{\text{oxygen}} \text{Oxyluciferyl-AMP} + \text{light} \quad (25b)$$

$$2\ \text{ADP} \xrightleftharpoons{\text{adenylate kinase}} \text{ATP} + \text{AMP} \quad (26)$$

$$\text{ATP} \xrightarrow{\text{apyrase}} \text{AMP} + \text{PP} \quad (27)$$

$$\text{AMP} \xrightarrow{\text{adenylate deaminase}} \text{IMP} + \text{NH}_3 \quad (28)$$

Reactions 24b and 28 involve a change in optical density at 340 mμ and 265 mμ, respectively; hence these reactions, and any others that may be linked to Reactions 24b and 28 by the appropriate enzymes, can be followed spectrophotometrically. The ATP-dependent luminescence of fire-fly extracts (Reactions 25a and 25b) has been used also for the assay of this nucleotide (77). Measurements of ATP based upon Reaction 24a are complicated by the fact that other nucleoside triphosphates can partially replace ATP (78); conversely, the fire-fly system is completely inactive with ADP, phosphocreatine, acetyl phosphate, ITP, UTP, GTP, and inorganic polyphosphates. Few specific assays are presently available for other nucleoside polyphosphates, but they undoubtedly could be devised with the aid of enzyme systems described below.

b. Biosynthesis. In most cells,* ATP synthesis occurs principally during anaerobic glycolysis or during the operation of the citric acid cycle. In the conventional glycolytic scheme (cf. Vol. I, Chapter 9), there are two steps where synthesis of ATP occurs:

$$\text{1,3-Diphosphoglycerate} + \text{ADP} \rightleftharpoons \text{3-Phosphoglycerate} + \text{ATP} \quad (29)$$

$$\text{Phosphoenolpyruvate} + \text{ADP} \rightleftharpoons \text{Pyruvate} + \text{ATP} \quad (30)$$

Reaction 29 actually represents a *net* synthesis of ATP from P_i since the acyl phosphate arose from the phosphate-dependent dehydrogenation of 3-phosphoglyceraldehyde:

$$\text{3-Phosphoglyceraldehyde} + \text{DPN}^+ + P_i \rightleftharpoons \text{1,3-Diphosphoglycerate} + \text{DPNH} + \text{H}^+ \quad (31)$$

* In systems lacking glycolysis, oxidative phosphorylation, or photosynthetic phosphorylation (e.g., certain anaerobic bacteria), the degradation of organic substrates often yields acyl thioesters, whose "energy-rich" bond is ultimately conserved as ATP (cf. Section III,B,1).

On the other hand, the phosphate group transferred in Reaction 30 was introduced earlier in the glycolytic scheme *via* an ATP-dependent reaction (cf. Eq. 24a). In the latter instance, the "energy-rich" bond has been conserved, rather than synthesized *de novo*.

In many systems, and especially in tissues of higher animals, the major site of ATP synthesis is the citric acid cycle. The complete combustion of one mole of pyruvate to CO_2 and H_2O would yield approximately 273 kcal. of energy (*27*). During the realization of this process via the citric acid cycle, approximately 14–15 moles of ATP are synthesized. ATP synthesis can occur only concomitantly with *oxidative* steps in the cycle (i.e., pyruvate → acetate, isocitrate → oxalosuccinate, α-ketoglutarate → succinate, succinate → fumarate, and malate → oxaloacetate). The term "oxidative phosphorylation" has been given to the process whereby P_i and ADP interact to form ATP during these oxidative steps (see pertinent reviews (*79–83a*). It should be noted that this process results in the *de novo* synthesis of ATP from "energy-poor" components.

The mechanism of oxidative phosphorylation is still obscure, due, at least in part, to the fact that the enzymes responsible for this process are found in most tissues to be associated with subcellular particles. These may be comminuted by treatment with digitonin (*84, 85*), ethanol (*86–87a*), or sonic oscillation (*88–89a*), to produce still smaller particles which retain the ability to carry out oxidative phosphorylation. It has been suggested that phosphorylation occurs at several points in the electron transport sequence intervening between the substrate and molecular oxygen. This view has been given strong support by the spectroscopic studies of Chance and his colleagues (*83*). Evidence has been obtained that there are 3 sites of phosphorylation in the sequence between DPNH and O_2: (1) between DPNH and a flavoprotein; (2) between cytochromes b and c; and (3) between cytochromes c and a. Furthermore, it has been noted that carefully prepared mitochondria ("tightly coupled" mitochondria) do not oxidize substrates until ADP is added to initiate oxidative phosphorylation. A hypothetical scheme showing oxidative phosphorylation at one stage in the electron transport sequence is given in Fig. 5. "A" and "AH_2" represent a typical substrate in the oxidized and reduced form. "DPNH ~ P" symbolizes an "energy-rich" adduct between P_i and DPNH, which is not oxidized by the flavoprotein system until the ~P is removed by transphosphorylation with ADP. The simplified net reaction for oxidative phosphorylation is depicted in Eq. 32. The resemblance of this reaction

$$\text{Energy} + P_i + \text{ADP} \rightarrow \text{ATP} \qquad (32)$$

to the reversal of ATP hydrolysis has led to speculation that ATPase may play a functional role in oxidative phosphorylation (*90, 91a*).

An interesting facet of oxidative phosphorylation is the observation that 2,4-dinitrophenol and a large variety of other substances (83) can "uncouple" the process, i.e., abolish the synthesis of ATP without impairing the oxidation. Like oxidative phosphorylation itself, the mechanism of the uncoupling process has not yet been elucidated (see, however, references *91b* and *91c* for discussions of this problem).

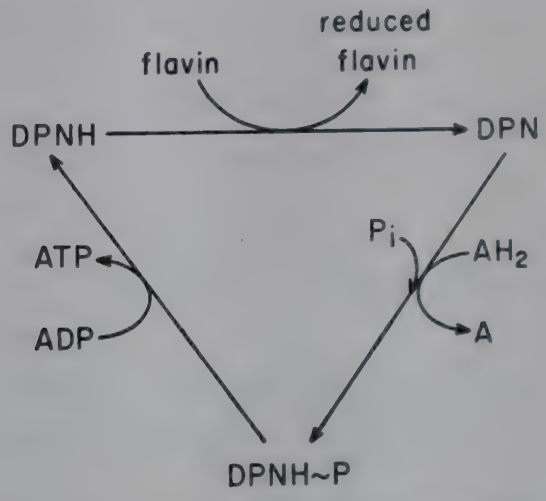


FIG. 5. Hypothetical representation of oxidative phosphorylation.

An important route of ATP synthesis in plants (*91d, 91e*) and photosynthetic bacteria (*91f, 91g*) occurs via a complex sequence of reactions termed "photosynthetic phosphorylation." The net process of ATP synthesis either may be coupled to the reduction of TPN (Eq. 32a), or be

$$2ADP + 2P_i + 2TPN^{+} + 4H_2O \xrightarrow{\text{light}} 2ATP + 2TPNH + 2H^{+} + O_2 + 2H_2O \quad (32a)$$

independent of TPN ("cyclic phosphorylation," Eq. 32b). The latter reaction is identical

$$n\text{ADP} + P_i \xrightarrow{\text{light}} n\text{ATP} \quad (32b)$$

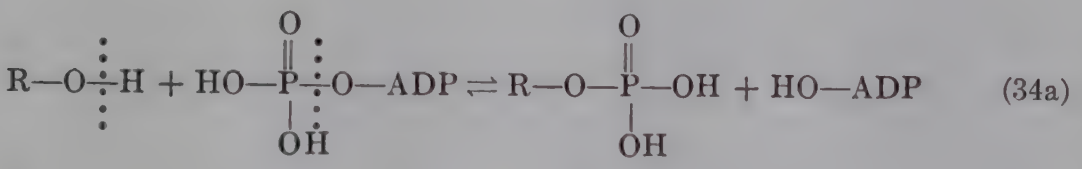
with Reaction 32, except that in "cyclic phosphorylation," the requisite energy is provided by light. The ATP produced by a combination of photosynthetic and oxidative phosphorylation is used, in part, for the dark-fixation of CO_2 by photosynthetic tissues (*91h–91k*). Reactions 32a and 32b have also been studied in chloroplast and chromatophore preparations with respect to intermediate electron carriers and the effect of uncoupling agents (*91l–91n*), but the detailed mechanism of photosynthetic phosphorylation, like oxidative phosphorylation, remains unknown.

Another mode of synthesis of ATP is via transphosphorylation reactions, as typified by Eq. 33:

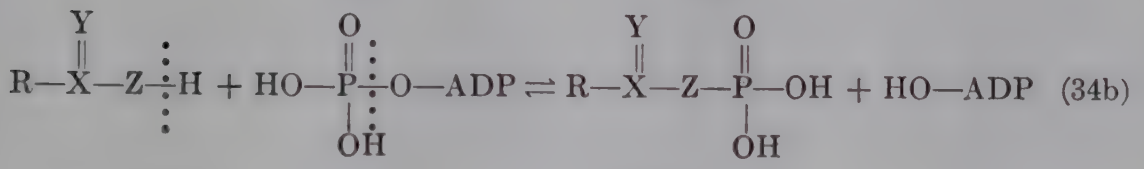
$$\text{X-phosphate} + \text{ADP} \rightleftharpoons \text{X} + \text{ATP} \qquad (33)$$

X-phosphate may be phosphocreatine or other *N*-phosphate compounds, phosphoenolpyruvate, glyceryl and other acyl phosphates, or, as shown in Reaction 23, other nucleoside triphosphates. These reactions involve the *transfer,* rather than the net synthesis, of an "energy-rich" linkage, and the equilibrium of Reaction 33 will depend, of course, upon the relative ΔF° of hydrolysis values for X-phosphate and ATP (cf. Table I).

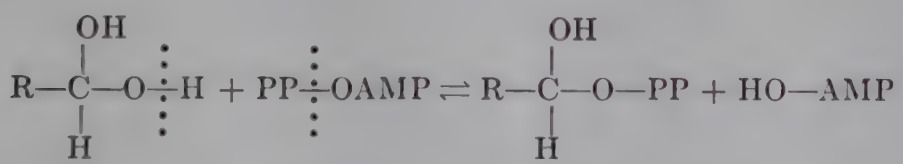
c. Function. At the molecular level, ATP functions as a phosphorylating agent (Eq. 34a,b), a pyrophosphorylating agent (Eq. 35), or an adenylating agent (Eq. 36). The site of bond cleavage in the reactants is indicated by the dotted line.



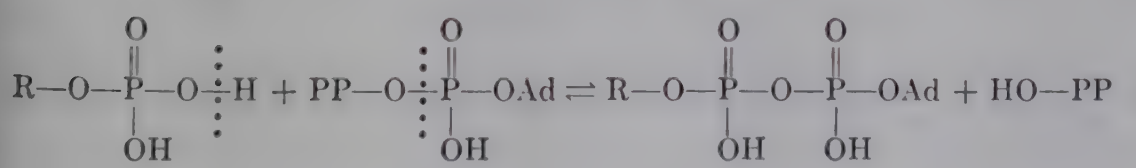
(34a)



(34b)



(35)



(36)

The first type of reaction (Eqs. 34a,b) is carried out by a multitude of *kinase* enzymes. When the substrate contains a susceptible alcoholic group,* the equilibrium position lies far to the right and the resulting phosphate ester is "energy-poor." In general, ROH represents a wide variety of sugars [see Table I in Kornberg's review (*50*)], although hydroxyamino acids or even water (as in the case of the enzyme,

* Exceptions to this generalization occur when the acceptor is a thioalcohol (cf. Section III,A,5) or a phosphate group. Examples of the latter reaction are the phosphorylation of AMP by ATP (Reaction 26) and the phosphorylation of 5-phosphomevalonic acid to the pyrophosphate during terpene synthesis (*92, 92a*).

ATPase) may serve as the phosphate acceptor. When the acceptor has the general structure

$$R-\overset{\overset{Y}{\|}}{X}-ZH$$

as in Eq. 34b, the resulting phosphorylated product is "energy-rich" and the equilibrium constant for the reaction is near unity. The acceptor in this reaction may be an acyl, phosphoryl, enol, or guanidyl group.

In the second type of reaction (Eq. 35), ATP donates its pyrophosphoryl group to the acceptor. The only well-established representative of this class of reactions is the interaction of ATP with ribose-5-phosphate to yield 5-phosphoribosyl-1-pyrophosphate plus AMP (*92b*). Since Eq. 35 is an equilibrium reaction, it follows that the aldehyde-phosphate bond in the product may be relatively "energy-rich."

Finally in Reaction 36, ATP is again cleaved in a manner similar to that in Eq. 35; except that the adenylic acid portion, rather than the pyrophosphate, is transferred to the acceptor. In all reactions of this type, the acceptor forms an "energy-rich" linkage with the phosphate group of the entering adenylate and the equilibrium constant is near unity. The acceptor may be the terminal phosphate of a mononucleotide, a fatty acid, an amino acid, or sulfuric acid [see Table II of Kornberg (*50*) for a summary of these reactions].

There is an additional class of ATP-dependent reactions, which is more complex than the preceding types inasmuch as 3 reactants are involved. Glutamine synthesis may be cited as an example [see Table V of Kornberg (*50*) for analogous reactions]:

$$\text{Glutamate} + NH_3 + \text{ATP} \rightleftharpoons \text{Glutamine} + \text{ADP} + P_i \qquad (37)$$

No phosphorylated or adenylated derivative of either glutamic acid or NH_3 has ever been detected as an intermediate in the reaction, and the detailed mechanism is unknown.

ATP has been implicated as the primary energy source for a number of physiological processes of which the following may be cited as representative examples: muscle contraction and motility of certain cells, transport of ions, water, and metabolites across membranes, renal function, and transmission of electric impulses in nerve tissue. It is becoming increasingly apparent that underlying each of these organ or cellular functions is a complex series of enzymatic reactions, one or more of which ultimately depends upon ATP as an energy source. At the present time, specific ATP-dependent reactions are being correlated with many physiological processes, but in most cases the exact nature and role of the enzymatic process is not yet understood.

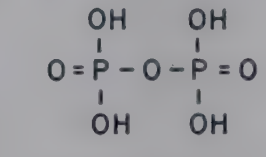
d. Distribution. ATP and other nucleoside 5′-polyphosphates have been identified unambiguously by chromatographic methods in acid extracts of the following tissues: rat organs (*62, 93–95b*), guinea pig mammary gland (*96*), human erythrocytes (*97–97c*), yeast (*98–100*), *Lactobacillus arabinosus* (*101*), eggs of the sea urchin *Psammechinus miliaris* (*102*), flight muscles of the housefly *Musca domestica* (*103, 104*), body wall of the earthworm *Lumbricus terrestris* (*105*), the pharynx muscle of the snail *Helix pomatia* (*106*), the sartorius muscle of the tortoise *Testudo graeca* (*106*), muscle of frog (*106*), squid (*107*), and carp (*108*), and plant tissues (*91i*). Other methods have been employed for the identification of ATP in a wide variety of tissues and cells, too numerous to document *in extenso.* There seems little doubt, however, that *ATP is the most important "energy-rich" compound in the entire phyletic sequence,* as evidenced by its seemingly universal distribution in animals, plants, and microorganisms. Even in living systems where ATP has not yet been specifically identified, the existence of any of the following metabolic sequences may be taken as presumptive evidence for the concomitant existence of ATP: glycolysis, pentose cycle, citric acid cycle, urea cycle, fatty acid oxidation and synthesis, protein synthesis, and nucleic acid synthesis. Furthermore, as illustrated in Section III of this chapter, the identification of other "energy-rich" compounds in a living system, again implies the presence of ATP because of known enzymatic reactions that involve the interaction of these compounds with ATP.

2. *Inorganic Polyphosphates*

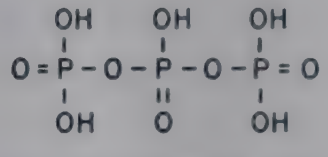
a. Preparation and Identification. The general term "polyphosphates" includes both linear and cyclic anhydrides of inorganic phosphate. The structures of the most commonly encountered low molecular weight members (pyrophosphate, tripolyphosphate, trimetaphosphate, and tetrametaphosphate) are shown in Fig. 6. Of these compounds, only pyrophosphate and tripolyphosphate have been investigated extensively. High molecular weight polymers, consisting of either linear or cyclic units, or mixed polymers, have been prepared synthetically. A linear polyphosphate is shown in Fig. 6. In biological systems the structural sub-unit of the high molecular weight polymers cannot be identified with certainty (*109, 110*); consequently, we shall use the term "polyphosphate" for all polymerized structures even though they may have been designated as metaphosphates in the original citations.

Polyphosphates are found in many cells, the first isolation from yeast being reported by Liebermann in 1888 (*111*). Standard fractiona-

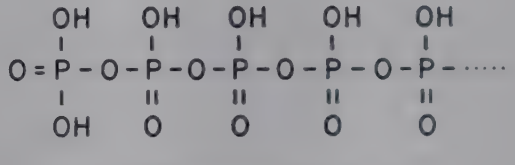
tion techniques of tissues and cells for phosphorus compounds yields two polyphosphate fractions (*112–118*). One fraction is extracted by *cold* trichloracetic acid ("soluble fraction"), whereas the other fraction is extracted only by treatment with alkali or *hot* trichloracetic acid ("insoluble fraction"). Selective precipitation techniques are required to separate the polyphosphates from the other phosphorus compounds



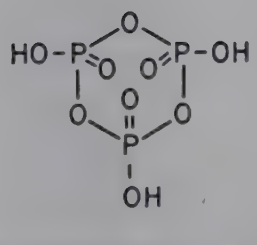
Pyrophosphoric acid



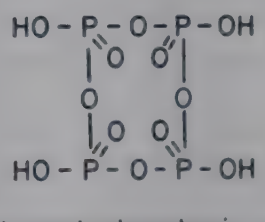
Tripolyphosphoric acid



Polyphosphoric acid



Trimetaphosphoric acid



Tetrametaphosphoric acid

FIG. 6. Inorganic polyphosphates.

found in these fractions. The lower molecular weight polyphosphates may be precipitated with Ba^{2+}, Mn^{2+}, or heavy metals; the more highly polymerized compounds are precipitated by Ba^{2+}, trichloracetic acid, or perchloric acid (*109*). Both the low molecular weight compounds and the highly polymerized phosphates may then be separated and identified by ion exchange (*119, 120*) or paper chromatography (*121, 122*).

Quantitative determinations of low molecular weight polyphosphates are based on measurement of P_i after acid hydrolysis or after enzymatic hydrolysis with pyrophosphatase, tripolyphosphatase, trimetaphosphatase, or tetrametaphosphatase (*123*). Certain of the low molecular weight compounds can also be determined by specific methods; for example, pyrophosphate and tripolyphosphate by reaction with $ZnSO_4$ (*109*), and pyrophosphate by precipitation with Mn^{2+} (*124*).

Highly polymerized phosphates are characterized by their precipitation with proteins at acid pH (*109*), by their complexing properties

with metal ions (*125, 126*), by high viscosity (*127*), and by their ability to induce metachromasy (*109*). The metachromatic reaction (i.e., the change in color of a dye such as toluidine blue from blue to pink or purple) has been widely used as a cytological method for locating polyphosphates in cells.* This method may be quantitated through the use of spectrophotometric techniques (*110, 115–115b*). Polyphosphate-containing granules of bacteria can be detected by means of electron microscopy (*128d, 128e*). Highly polymerized polyphosphates can also be determined by measuring the release of P_i, or by measuring changes in viscosity or sedimentation rate (*127*) after acidic or enzymatic hydrolysis (*109, 122, 123*).

b. Biosynthesis. Pyrophosphate does not appear to be synthesized, as such, from P_i, but is formed as a by-product from a wide variety of pyrophosphorylase reactions (*50*) (cf. Eq. 36). Specific examples include the synthesis of many coenzymes (DPN, CoA, flavin adenine dinucleotide, cytidine diphosphate choline, uridine diphosphate glucose), of acyl adenylates, of amino acyl adenylates, and of "active sulfate." Pyrophosphate is also formed by the apyrase-catalyzed hydrolysis of ATP (*129*), and from tripolyphosphate as described below (Reaction 38 in reverse).

Tripolyphosphate, trimetaphosphate, and tetrametaphosphate may appear as intermediates in the degradation of highly polymerized polyphosphate (*122, 123, 130*). Tripolyphosphate (PPP) may also be synthesized by adenylate kinase via Reaction 38 (*131*)

$$PP + ADP \rightleftharpoons PPP + AMP \tag{38}$$

or from the splitting of deoxyguanosine triphosphate (and to a lesser extent guanosine triphosphate) by an enzyme system from *Escherichia coli* (*131a*):

$$\text{Deoxy-GTP} \rightarrow \text{Deoxyguanosine} + PPP \tag{38a}$$

The linear polymer, tetraphosphate, may be synthesized in yeast by the addition of P_i to cyclic trimetaphosphate (*131a*).

High molecular weight polyphosphate is rapidly accumulated (*113–115, 132*) when phosphate-starved yeast is placed into a medium containing phosphate. This accumulation is accompanied by the appearance of basophilic or metachromatic granules called "volutin." These granules were first isolated by Wiame (*113, 115*), who termed them "metaphosphate," but subsequent work has shown that this material is,

* Metachromasia results from the polymerization of the dye (*128*) which may be induced not only by polyphosphates, but also by glycoproteins (*128a*) and ribonucleic acid (*128b,c*), thus indicating that staining properties cannot be used as the sole criterion for the identification of polyphosphates.

in fact, highly polymerized polyphosphate (*110*). The formation of "volutin" by yeast and bacteria requires an oxidizable substrate, phosphate, and potassium ions (*133*), and is inhibited by iodoacetate, fluoride, azide (*115*), and dinitrophenol (*128b*).

It has been reported (*115–117, 128b, 132–135c*) that yeasts, molds, and bacteria contain both a "soluble fraction" and an "insoluble fraction" of polyphosphates regardless of growth conditions. These fractions have been further distinguished from each other by their metabolic behavior. The amount of the "insoluble fraction" was reduced during nucleic acid synthesis (*115, 134–136*); this was followed by the depletion of the "soluble" polyphosphate. P^{32}-labeled orthophosphate was incorporated rapidly only into the "insoluble fraction." It has been suggested recently (*110, 118*) that the "soluble fraction" is composed of low molecular weight material on the basis of its physical and chemical properties. In view of the noninterconversion of the two fractions and the above metabolic differences, it appears possible that they are formed by independent mechanisms (*110*).

Some evidence has been obtained that the formation of polyphosphates by yeast (*137–139a*) is the result of Reaction 39, catalyzed by "metaphosphate kinase":

$$x\text{ATP} + (PO_3)_n \rightleftharpoons x\text{ADP} + (PO_3)_{n+x} \tag{39}$$

Kornberg *et al.* (*110*) have studied this reaction more extensively with a purified enzyme from *Escherichia coli*. These investigators found that the reaction yielded trichloracetic acid-insoluble material, formed by polymerization of the terminal phosphate of ATP. Lower molecular weight compounds were not produced; thus, it is possible that *E. coli* has only one of the two polyphosphate-forming mechanisms suggested for yeast. The product also: (*a*) induced metachromasy; (*b*) was not dialyzable; (*c*) formed an acid-insoluble complex with protein; (*d*) was labile to acid and base; (*e*) was bound firmly by anion exchange resins; and (*f*) was resistant to nucleases. Furthermore, this enzyme could utilize PP in place of ATP in Reaction 39, although at a slower rate (*50*). The pyrophosphate reaction is shown in Eq. 40.

$$\text{PP} + (PO_3)_n \rightleftharpoons P_i + (PO_3)_{n+1} \tag{40}$$

It is possible that Reaction 40 could also occur in yeast, thereby accounting for the rapid incorporation of P^{32}-labeled P_i into the "insoluble fraction."

A further study (*140*) of the polyphosphate-synthesizing enzyme from *E. coli* showed that polyphosphate, prepared by chemical or enzymatic synthesis, may be utilized in the quantitative phosphorylation

of ADP to ATP, according to Reaction 41. In contrast to Reaction 39, this process did not require a primer.

$$n\text{ADP} + (PO_3)_n \rightleftharpoons n\text{ATP} \qquad (41)$$

ADP could not be replaced by the diphosphates of cytidine, uridine, or guanosine, or by AMP.

Polyphosphate synthesis according to Reaction 41 was also demonstrated in extracts of *Corynebacterium diphtheriae* (*140*). This result is not unexpected since this organism has long been known to accumulate large amounts [approx. 30% of the cell weight (*141*)] of "volutin."

The mechanism of polyphosphate synthesis in cells of higher plants (*109*) and algae (*142*) has not been investigated. However, the formation of maximum amounts of polyphosphate in *Chlorella* (*142*) was found to occur in the light and in the absence of ATP-utilizing reactions such as CO_2 fixation; in the dark, the amount of polyphosphate was decreased. It is possible that polyphosphates are formed in plant cells by a reaction similar to Eqs. 39 or 41.

Nickerson (*143*) found that yeasts are able to polymerize glucose and phosphate at the same rate and that both polymerizations are inhibited to the same extent by addition of dinitrophenol and azide. Reaction 42 was proposed as a

$$n(\text{Glucose-1-phosphate}) \rightleftharpoons \text{Glycogen} + (PO_3)_n \qquad (42)$$

mechanism for polyphosphate formation on the assumption that the insolubility of the polyphosphate product would shift the otherwise unfavorable equilibrium.

c. Function. Changes in content of the insoluble polyphosphate pool during nucleic acid synthesis (*115–117, 128b, 134–135c*) and cell division (*132, 134, 135*) suggest that these compounds function as phosphate reservoirs; release of P_i could be effected by the action of specific depolymerizing enzymes. Such enzymes have been found in yeasts (*122*), in animal tissues (*123*), in serum (*144*), in molds (*145, 146*), in plants (*146a*), and in bacteria (*110, 127, 140, 146, 146b*). Enzymes capable of hydrolyzing tripolyphosphate, trimetaphosphate and tetrametaphosphate are also found in these tissues and cells (*109, 146c*), and pyrophosphatase (*147*) has been found in almost all tissues.

The "energy-rich" nature of polyphosphates was first established by Meyerhof's group (*40*) from measurements of the ΔF and ΔH for the hydrolysis of metaphosphate. It is obvious that Reactions 39 and 41 provide a means for mobilizing polyphosphate for energy purposes; for example, ATP synthesis via these reactions has been coupled to the phosphorylation of glucose (*140*) and glycerol (*148*). Under unfavorable growth conditions, or at certain stages in the growth cycle, the

accumulation of ATP might give rise to polyphosphate via the above reactions. Such an accumulation of polyphosphate has been observed frequently (*115, 118, 128b, 134–136, 142, 148–150b*). Upon resumption of growth, ATP utilization would yield ADP, which could then be recycled to ATP by reversal of Reaction 39 or 41. The function of polyphosphates would thus be analogous to that of the *N*-phosphate compounds, phosphocreatine and phosphoarginine, in animal tissues. It is of interest to note that cells capable of accumulating large amounts of polyphosphate—i.e., yeasts, molds, plant cells, and bacteria—do not contain the *N*-phosphate compounds.

The "energy-rich" bond of pyrophosphate could also be preserved through the concerted action (*50*) of the enzymes mediating Reactions 39 and 40. Summation of these equations yields the net reaction (Eq. 43):

$$PP + ADP \rightleftharpoons P_i + ATP \tag{43}$$

Some evidence has been obtained (*50*) that a single enzyme, adenylate kinase, can bring about Reaction 43.

As stated earlier, some tissues are able to carry out Reaction 38 thus conserving the "energy-rich" bond of tripolyphosphate; the rate of this reaction, however, is very low. Although the affinity of myosin for tripolyphosphate is poor (*151*), this compound can cause a slight contraction of myofibrils (*152*). No evidence has been obtained for the participation of other low molecular weight polyphosphates as "energy-rich" reservoirs.

Polyphosphates may also function to control cellular metabolism (*118, 135a, 136, 153*) by virtue of their ability to complex with cations and proteins (*125, 126*). Inactivation or removal of essential enzymes, or binding of cations required for enzyme activation, would obviously influence the course of metabolism. It has been shown (*154*), for example, that metaphosphate inhibits hexokinase both *in vivo* and *in vitro* by virtue of its ability to bind Mg^{2+}.

d. Distribution. Schmidt (*109*) has reviewed the occurrence of polyphosphates in yeasts, plant cells, molds, and many bacteria. Aside from pyrophosphate, and possibly small amounts of tripolyphosphate formed via Reaction 38, polyphosphates have not been found in animals with the exception noted below. This is surprising in view of the widespread distribution in animal tissues of enzymes capable of depolymerizing polyphosphates (*123, 144*).

Niemerko and co-workers (*155–157*) found that 2–4% of the excreta of the larvae of the wax moths *Galleria mellonella* and *Achroia grisella* consists of polyphosphate. These observations have been confirmed and,

in addition, varying amounts of polyphosphate, pyrophosphate, and tripolyphosphate were found (*158, 159*). Production of the polyphosphates in the intestinal lumen by the action of bacteria seems to have been ruled out, at least as the sole mechanism of formation, since urine collected directly from the Malpighian tubules and the meconium of the imago also contained polyphosphates. An accumulation of pyrophosphate has also been reported to occur in the ejaculatory duct of the hawk-moth, *Celerio euphorbiae* (*160, 160a*), and to be transferred to the female during copulation (*160a*). Experiments with labeled ATP showed that pyrophosphate was produced in the male organ by apyrase action (Reaction 27) and accumulated there owing to the absence of pyrophosphatase (*160b*).

3. *Acyl Phosphates*

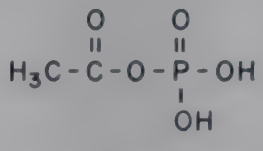
a. Acetyl and Other Carboxyl Phosphates:

(*1*). *Preparation and identification.* The structure of a representative carboxyl phosphate, acetyl phosphate, is given in Fig. 7. Other carboxyl phosphates may be formed by bacteria, plants, and animal tissues. For example, butyryl (*161, 162*), propionyl (*162, 163*), carbamyl (*164–165a*), and aspartyl (*166*) phosphates have been isolated, and some evidence concerning the possible existence of carbonyl (*167*), succinyl (*163, 168, 169*), and glutamyl (*170*) phosphates has been presented. In the following discussion, attention will be directed primarily to acetyl phosphate because it is the most widely encountered and best-documented member of this class of "energy-rich" compounds.

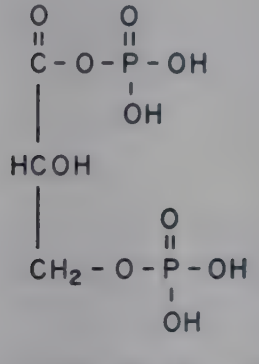
Acetyl phosphate was first isolated by Lipmann (*171, 172*), as an end product in the phosphate-dependent oxidation of pyruvate by *Lactobacillus delbrueckii* (*173*). Analysis of other phosphate-dependent pyruvate degradations indicated that several bacterial systems were capable of producing acetyl phosphate (*174–176*). Such degradations were termed "phosphoroclastic reactions" (*172, 177*). Chemical synthesis of acetyl phosphate may be accomplished by the reaction of: (*a*) acetyl chloride with silver dibenzyl phosphate (*178*); (*b*) acetyl chloride with silver dihydrogen phosphate (*179*); (*c*) isopropenyl acetate with orthophosphoric acid (*180, 181*); and (*d*) acetic anhydride with orthophosphate in aqueous pyridine (*182*). The fourth method is essentially quantitative and has been used, after slight modification (*110*), to prepare P^{32}-labeled acetyl phosphate. The second and fourth methods have also been used to prepare propionyl (*179*), butyryl (*179*), and succinyl (*169, 179*) phosphates.

The lability of acyl phosphates in acid molybdate has been utilized

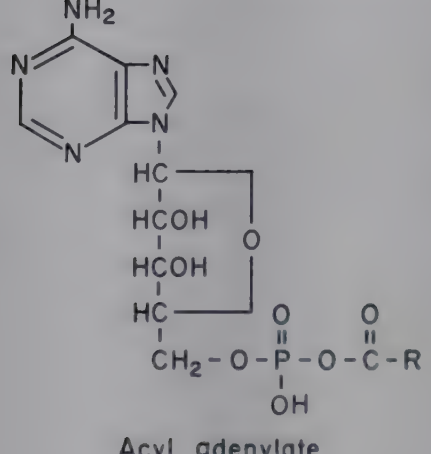
for the quantitative estimation of acetyl phosphate (*179*). A more accurate determination is based on the reaction of acetyl phosphate with hydroxylamine at neutral pH (*183*); the resulting hydroxamic acid gives a colored complex with Fe^{3+}. The hydroxamates of acyl phos-



Acetyl phosphate



1,3-Diphosphoglycerate



Acyl adenylate

FIG. 7. Acyl phosphates.

phates may also be separated by paper chromatography (*162*).

(*2*). *Biosynthesis.* Acetyl phosphate may be formed by the phosphorylation of acetate by ATP (*162, 172, 184*), as shown in Reaction 44.

$$\text{Acetate} + \text{ATP} \rightleftharpoons \text{Acetyl phosphate} + \text{ADP} \qquad (44)$$

The enzyme mediating this reaction, *acetokinase*, has been purified by Rose *et al.* (*185, 186*), and shown to require Mg^{2+} or Mn^{2+}, and to be inhibited by sulfhydryl reagents. ATP can be replaced by ITP and IDP, but not by ADP. The formation of acetyl phosphate via Reaction 44 is generally measured with hydroxylamine as a "trapping" agent, since

the equilibrium lies far to the left. Propionate can also be phosphorylated in the acetokinase system, but only at a low rate compared to acetate.

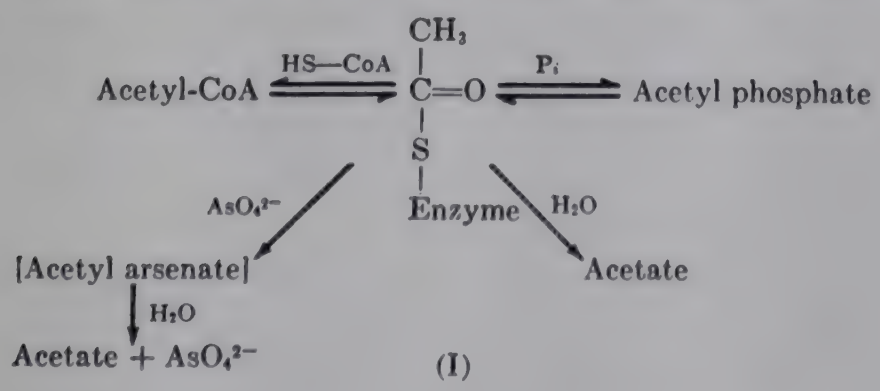
Acetyl phosphate is also synthesized from acetyl-CoA by the action of the enzyme phosphotransacetylase (*162, 187–191a*), as shown in Reaction 45:

$$\text{Acetyl-CoA} + P_i \rightleftharpoons \text{Acetyl phosphate} + \text{CoA} \qquad (45)$$

If P_i is replaced by arsenate, acetyl arsenate is formed, which then undergoes spontaneous hydrolysis. The "arsenolysis" of acetyl phosphate, on the other hand, requires the formation of acetyl-CoA as an intermediate; this reaction has been used as an assay for CoA (*189*). Propionyl-CoA will react more slowly than acetyl-CoA with phosphotransacetylase, whereas butyryl-CoA reacts only at a barely perceptible rate. The CoA derivatives of formate, lactate, and the higher fatty acids are completely inactive (*189*).

Acetyl phosphate may also be formed from the degradation of pyruvate. In some systems, pyruvate oxidation yields acetyl lipoate or acetyl-CoA [reviewed by Gunsalus *et al.* (*192*)]. The latter compound, in the presence of P_i and phosphotransacetylase, can then give rise to acetyl phosphate. However, the oxidation of pyruvate by *L. delbrueckii* requires P_i, but not CoA (*193*), and acetyl phosphate appears to be formed in the primary reaction. The term "phosphoroclastic reaction" (*177*) should be reserved for systems of the latter type.

In mammalian tissues, acetyl phosphate can be formed by the oxidation of acetaldehyde by 3-phosphoglyceraldehyde dehydrogenase (*194*), although the rate of this reaction is far lower than the usual oxidation of 3-phosphoglyceraldehyde. 3-Phosphoglyceraldehyde dehydrogenase also mediates the exchange of P_i into acetyl phosphate, the arsenolysis and hydrolysis of acetyl phosphate, and a transfer of the acetyl radical to CoA or glutathione (*195–197*). The formation of a thioester linkage between the enzyme and the acyl group, followed by phosphorolysis or transfer reactions, as shown in Scheme I, has been postulated to account for these findings (*197*). A similar mechanism has been sug-



(I)

gested to explain the formation of acetyl phosphate from the scission of xylulose-5-phosphate (*198*); and could also be invoked for pyruvate degradations not requiring lipoic acid. In the latter instance, phosphorolysis or transfer reactions would follow oxidation of the enzyme-bound "acetaldehyde-diphosphothiamine" complex, yielding acetyl phosphate or acetyl-CoA.

(*3*). *Function.* Acetyl phosphate has two major functions: it is a reservoir of "energy-rich" phosphate, and is a precursor of acetyl-CoA. The "energy-rich" bond is made available for ATP synthesis by reversal of Reaction 44. Especially in bacteria, acetyl phosphate and the acetokinase system may be an important energy reserve for ATP synthesis.

Phosphotransacetylase facilitates an isoenergetic interconversion of acetyl phosphate and acetyl-CoA. The latter compound is required for numerous synthetic reactions, as will be discussed in Section III,B,1. It is obvious also that Reaction 45 represents not only a mechanism for conserving the "energy-rich" bond of acetyl-CoA, but also for releasing CoA for further reactions.

In addition to generating ATP directly, acetyl phosphate can replace ATP as the phosphorylating agent in certain metabolic reactions (*199*), as illustrated by the example in Eq. 46.

$$\text{Acetyl phosphate} + \text{Glycerol} \rightleftharpoons \text{Glycerol phosphate} + \text{Acetate} \qquad (46)$$

Transfer reactions of this type are frequently carried out by phosphatases [reviewed by Axelrod (*200*)].

In bacterial systems, the phosphorolytic cleavage of acyl-enzyme complexes conserves the energy derived from an oxidative step. In mammalian tissues, which lack both acetokinase and phosphotransacetylase, acetyl phosphate cannot be used to generate ATP. Furthermore, acetyl phosphate is rapidly hydrolyzed in mammalian tissues by an acyl phosphatase (*177, 201–202a*), which, unlike the enzymes mediating Reaction 46, is incapable of transferring the phosphate group to any acceptor other than water. However, thiolysis of acyl-enzyme complexes by CoA (for example, in the degradation of pyruvate by bacterial or animal systems) conserves the energy of oxidation.

Other carboxyl phosphates participate, or have been postulated as intermediates, in various reactions: carbamyl phosphate* in the synthe-

* Carbamyl phosphate (CP) is synthesized in mammalian and frog liver by the reaction (*164a*):

$$2\ \text{ATP} + \text{NH}_3 + \text{CO}_2 \xleftarrow{\text{acetyl glutamate}} \text{CP} + 2\ \text{ADP} + \text{P}_i \qquad (46a)$$

while in bacteria (*164b*) a slightly different system is found:

$$\text{ATP} + \text{NH}_3 + \text{CO}_2 \rightleftharpoons \text{CP} + \text{ADP} \qquad (46b)$$

sis of citrulline (*164–165a*) and pyrimidines (*165a*), β-aspartyl phosphate in the conversion of aspartate to homoserine (*166*), and carbonyl phosphate in the oxidation of propionate (*167*) by mammalian tissues. The participation of glutamyl phosphate in glutamine synthesis is uncertain (*170*), as is the existence of succinyl phosphate as an intermediate in α-ketoglutarate oxidation (*168, 169*) or in succinate decarboxylation (*163*).

(*4*). *Distribution.* Acetyl phosphate (and also propionyl and butyryl phosphate) has been found primarily in microorganisms (*177, 183, 186–191*), although the occurrence of phosphotransacetylase in *Phaseolus radiatus* (*191a*) suggests that acetyl phosphate may be present also in higher plants. While this compound can be formed by mammalian tissues and yeast by the action of 3-phosphoglyceraldehyde dehydrogenase (*194–197*), acetyl phosphate is not accumulated because of the almost universal presence of acyl phosphatases in these cells (*76, 201, 202*). The other carboxyl phosphates mentioned above have been studied largely with specific mammalian tissues, but their participation in several key metabolic processes would suggest a general distribution in nature.

b. 1,3-Diphosphoglycerate:

(*1*). *Preparation and identification.* 1,3-Diphosphoglycerate, whose structure is shown in Fig. 7, was originally isolated as the end-product of the P_i-dependent oxidation of 3-phosphoglyceraldehyde (*203, 204*) (cf. Eq. 47).

$$\text{3-Phosphoglyceraldehyde} + \text{DPN}^+ + P_i \rightleftharpoons \text{1,3-Diphosphoglycerate} + \text{DPNH} + H^+ \quad (47)$$

If alcohol dehydrogenase and acetaldehyde are added, DPN is regenerated (Reaction 48) and the equilibrium of Reaction 47 is shifted to the right.

$$\text{DPNH} + H^+ + \text{Acetaldehyde} \rightleftharpoons \text{DPN}^+ + \text{Ethanol} \quad (48)$$

With pure enzyme preparations, this coupled reaction can be used for the synthesis of 1,3-diphosphoglycerate in good yield (*205*).

An enzymatic assay for 1,3-diphosphoglycerate is based on reversal of Reaction 47 and involves a spectrophotometric measurement of DPNH disappearance (*203–205*).

(*2*). *Biosynthesis.* 1,3-Diphosphoglycerate is synthesized solely by Reaction 47, as shown above. The substrate for this reaction, 3-phosphoglyceraldehyde, arises normally by glycolysis and the pentose cycle or, to a lesser extent, by direct cleavage of xylulose-5-phosphate. 3-Phosphoglyceraldehyde dehydrogenase, the enzyme-mediating reaction (Eq. 47), has been crystallized from yeast (*206, 207*) and muscle (*208,*

209) and purified from plant material (*210–212*). All these enzymes require Mg^{2+}, are inhibited by sulfhydryl reagents, and require DPN, except the enzyme from *Chlorella pyrenoidosa* and from spinach leaves, which is TPN-specific. Purified enzyme preparations can oxidize glyceraldehyde, acetaldehyde, propionaldehyde, and butyraldehyde (*194*) to the corresponding acyl phosphates.

(*3*). *Function.* The transphosphorylation of ADP from 1,3-diphosphoglycerate is catalyzed by the enzyme *phosphoglycerate kinase*, as shown in Reaction 49.

$$\text{1,3-Diphosphoglycerate} + \text{ADP} \overset{Mg^{2+}}{\rightleftharpoons} \text{3-Phosphoglycerate} + \text{ATP} \qquad (49)$$

Phosphoglycerate kinase has been crystallized from yeast (*33*) and is specific for all components shown in Reaction 49. The equilibrium of the reaction lies far to the right. The formation of ATP via Reaction 49 thus provides a mechanism for utilizing the energy gained in the preceding oxidation (Eq. 47).

(*4*). *Distribution.* The enzymes responsible for Reactions 47 and 49 have been reported in a wide variety of microorganisms, plants, and animals. 1,3-Diphosphoglycerate, per se, would not be expected to accumulate in large quantities because of its instability in aqueous solutions and its active role in metabolism. It would be anticipated, however, that this compound would be present, albeit possibly in trace amounts, in *all* cells that are capable of glycolysis or that possess the enzymes of the pentose cycle and 3-phosphoglyceraldehyde dehydrogenase.

c. Acyl Adenylates:

(*1*). *Preparation and identification.* Acyl adenylates have been invoked as intermediates in the ATP-dependent "activation" of acetic (*213, 214*), butyric, and higher aliphatic fatty acids (*215–219*), amino acids (*220–222*), benzoic acid (*223–225*), phenylacetic acid (*224*), sulfuric acid (*44c, 226–232a*), selenic, molybdic, and sulfurous acids (*229–229b*), lipoic acid (*233*), pantothenic acid (*234*), luciferin (*235, 235a*), and ammonia (*235b,c*). The general structure of an acyl adenylate is shown in Fig. 7.

Studies of the above enzyme systems have been greatly facilitated by the advent of specific chemical methods for the synthesis of acyl adenylates. These include: (*a*) condensation of the acid anhydride with AMP in the presence of pyridine (*182, 214, 216, 219, 235d*); (*b*) condensation of the free acid and AMP in the presence of carbodiimide as the dehydrating agent (*215, 236–236c*); and (*c*) reaction of the acyl chloride with the silver salt of AMP (*213, 214, 221, 237, 237a*). Enzy-

matic synthesis of acyl adenylates has been reported for tryptophanyl-AMP (*238–238c*), valyl-AMP (*239*), and seryl-AMP (*239a*).

Acyl adenylates may be identified by paper chromatography (*215*) or paper ionophoresis (*238*), by reaction with hydroxylamine (*214–216*), and by the activity of these compounds in appropriate enzymatic reactions.

(*2*). *Biosynthesis.* Studies on the mechanism of acetate activation by an enzyme purified from yeast led Berg (*213, 214*) to suggest that the over-all reaction shown in Eq. 50 actually occurs in two steps (Eqs. 51 and 52).

$$\text{Acetate} + \text{ATP} + \text{CoA} \rightleftharpoons \text{Acetyl-CoA} + \text{AMP} + \text{PP} \quad (50)$$

$$\text{Acetate} + \text{ATP} \rightleftharpoons \text{Acetyl-AMP} + \text{PP} \quad (51)$$

$$\text{Acetyl-AMP} + \text{CoA} \rightleftharpoons \text{Acetyl-CoA} + \text{AMP} \quad (52)$$

Although it was not possible to demonstrate the net synthesis of acetyl-AMP via Reaction 51, chemically synthesized acetyl-AMP reacted with the purified enzyme in a manner consistent with Reactions 51 and 52. Thus, acetate and ATP were produced from acetyl-AMP and PP, whereas acetyl-CoA and AMP were the products from acetyl-AMP and CoA (*213, 214*). Other acyl adenylates prepared by chemical synthesis have been shown to react with the corresponding activating enzymes in a comparable manner (*215–217, 237, 237b*). Further support for the above formulation has been provided by studies with O^{18}-labeled substrates (*240, 241*).

Several explanations have been proposed to account for the failure of acyl adenylates to accumulate when the enzyme is incubated with the acid and ATP. It has been suggested, for example, that these substances exist only as enzyme-bound complexes (*214–216, 242, 243*), or that deacylation of the intermediate may occur (*215, 216, 219*). Recently it has been pointed out that acyl adenylates are much more "energy-rich" (by about 6 kcal./mole) than ATP and, thus, that the equilibrium of Reaction 51 lies far to the left (*47*). The isolation of small amounts of aminoacyl adenylates could be explained, therefore, in terms of dissociation from an enzyme, inactivity of a deacylase, or a shift in the equilibrium by use of large amounts of reactants.

Presumably, the biosynthesis of acyl adenylates from a variety of acids proceeds by way of reactions similar to Eq. 51. Reaction 52, also, is typical of the various acyl adenylates, except that CoA may be replaced by other acceptors.* For example, pantoyl-AMP reacts with

* While it had been postulated originally that the "activation" of CO_2 involved carbonyl-AMP (*244*), which then reacted with a branched-chain fatty acid, later work (*244a*) has indicated that "active CO_2" is more probably an "energy-rich" CO_2-biotin complex (*244b–e*).

β-alanine to give pantothenate (*234*), β-alanyl-AMP reacts with histidine to yield carnosine (*245*), and "active sulfate" may be transferred to phenols, steriods, hexosamines, galactose, etc., (*232, 232a*).

It should be noted that "active sulfate" (PAPS, below) is formed in two steps, i.e.,

$$SO_4^{2-} + ATP \rightleftharpoons \text{Adenosine-5'-phosphosulfate (APS)} + PP \quad (52a)$$

$$APS + ATP \rightleftharpoons \text{3'-Phosphoadenosine-5'-phosphosulfate (PAPS)} + ADP \quad (52b)$$

The properties of the enzyme, ATP sulfurylase, which mediates Reaction 52a have been determined, and the enzyme has been partially purified (*44c, 229b*). APS has also been synthesized chemically (*245a, b*). In addition to being transferred to the acceptors listed above, PAPS (and also APS) has been implicated as an intermediate in the enzymatic reduction of sulfate (*245c–245e*).

(3). *Function.* The role of acetyl-AMP in acetate activation has been indicated above; other acyl adenylates may be formed in similar reactions. Special mention should be made of the role of amino acyl adenylates in protein synthesis. Amino acid activating systems were first encountered in extracts of rat liver (*220, 243*), bacteria (*221*), and yeast (*222*). Subsequent investigation revealed the widespread distribution of these systems in many bacteria (*237, 246–248b*), vertebrate tissues (*239, 249–252*), invertebrate tissues (*253*), yeast (*254*), molds (*247*), plants (*247, 252, 255*), and protozoa (*256*). The "activation" of an amino acid is postulated to occur via the generalized Reaction 53:

$$\text{R—CH(NH}_2\text{)—COOH} + ATP \rightleftharpoons \left[\text{R—CH(NH}_2\text{)—C(=O)—AMP}\right] + PP \quad (53)$$

The bracket indicates an enzyme-bound intermediate. This formulation is supported by the accumulation of amino acyl hydroxamates in the presence of hydroxylamine, and by the amino-acid-dependent exchange of P^{32} into ATP.

Later investigations revealed that a complete mixture of approximately 20 of the common amino acids gave maximum exchange (*221, 243, 246, 248, 254, 257, 258, 258a*) of PP into ATP, thus suggesting the existence of a wide variety of individual amino-acid-activating enzymes. Specific enzymes for tryptophan (*259*), tyrosine (*252–252b*), and methionine (*222*), were subsequently obtained in highly purified form.

It is believed that the aminoacyl group of the adenylate formed via Reaction 53 is transferred first to the 2'- or 3'-hydroxyl group of the terminal nucleotide of a soluble ribonucleic acid (*260–261c*), and then to a microsomal ribonucleoprotein [reviewed by Zamecnik *et al.* (*239*)].

The latter step specifically requires ATP and GTP (*262*), although the linkage between the acyl group and the soluble RNA is believed to be "energy-rich" (*261b, 262a*). This sequence of events, shown in Fig. 8, has been mapped out chiefly through the use of C^{14}-labeled amino acids. E_1, E_2, and E_3 indicated in Fig. 8 are separate enzymes, RNA and RNP are ribonucleic acid and ribonucleoprotein particles, respectively, and [a.a.]* is a labeled amino acid.

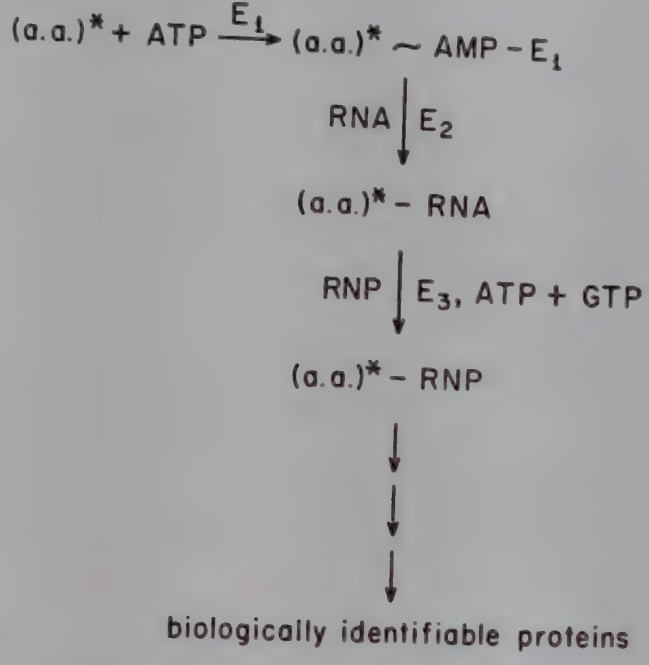


FIG. 8. Proposed sequence of events in protein synthesis.

(*4*). *Distribution.* Although the existence of *free* acyl adenylates is still in doubt, it appears reasonably certain that these compounds, or some very similar entities, arise during the course of activation reactions* similar to that portrayed in Eq. 50. Acyl adenylates appear to serve as transient intermediates in three-component reactions, where the initial "energy-rich" character of ATP is transmitted through, and conserved in, the intermediate. From the widespread occurrence of "activating" enzymes, it would appear likely that acyl adenylates are present in all animal, microbial, and plant cells.

4. *Enol Phosphate: Phosphoenolpyruvate*

a. Preparation and Identification. Phosphoenolpyruvate (PEP), whose structure is shown in Fig. 9, was first isolated from mammalian muscle (*263*). The compound can be separated from other phosphate

* No evidence has been obtained for the occurrence of comparable acyl adenylates in "activation" reactions where ADP and P_i are the end-products (e.g., those involving succinate, glutamate, formate, etc.). For these latter reactions, phosphorylated intermediates seem more probable.

esters and identified by chromatography on paper (264, 265) or on i exchange resins (266). Chemical synthesis of PEP may be achieved b phosphorylation of pyruvate in quinoline with $POCl_3$ (267–270), or b phosphorylation of β-chlorolactate in dimethylaniline with $POCl_3$, fo lowed by addition of alcoholic KOH (271, 272). In either synthes

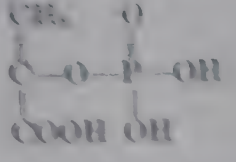


FIG. 9. Phosphoenolpyruvate.

purification is obtained by precipitation with Ba^{++}, or by passa through an ion exchange column (273). The analogous compou vinyl phosphate ($CH_2 = CHOPO_3H_2$) has been synthesized from vi acetate and P_i (273a).

Quantitative determinations are based on measurements of P, al addition of acid (274), mercury salts (274), or hypoiodite (275). P can also be detected by its absorption (276) of light at approx. 2 mμ. A convenient spectrophotometric assay utilizes the enzyme *py vate phosphokinase* (Eq. 54) coupled with lactic dehydrogenase a DPNH (Eq. 55).

$$\text{PEP} + \text{ADP} \rightleftharpoons \text{Pyruvate} + \text{ATP}$$

$$\text{Pyruvate} + \text{DPNH} + \text{H}^+ \rightleftharpoons \text{Lactate} + \text{DPN}^+$$

Similar assays, using the enzymes *PEP carboxylase* or *oxaloace carboxylase* (described below) coupled with malic dehydrogenase DPNH, might also be devised.

b. Biosynthesis. PEP appears as an intermediate in glycolysis, be produced from 2-phosphoglyceric acid (2-PGA) by the action of Mg^{++}-dependent enzyme, enolase, as shown in Reaction 56:

$$\text{2-PGA} \rightleftharpoons \text{PEP} + \text{H}_2\text{O}$$

Enolase has been crystallized and the kinetics of Reaction 56 have b studied extensively (277).

The decarboxylation of oxaloacetate by oxaloacetate carboxylase the presence of either GTP or ITP can also yield PEP, as shown Reaction 57.

$$\text{Oxaloacetate} + \text{GTP (or ITP)} \xrightarrow{Mg^{++}} \text{PEP} + \text{CO}_2 + \text{GDP (or IDP)}$$

Studies of this reaction stem from the initial observations on car dioxide fixation by heterotrophs. As originally formulated by Wood Werkman [reviewed by Werkman and Wood (278) and by Utter

[ood (279)], the fixation reaction involved carbon dioxide and free ruvate (**Reaction 58**).

$$CO_2 + \text{Pyruvate} \rightleftharpoons \text{Oxaloacetate} \qquad (58)$$

some bacteria, notably *Micrococcus lysodeikticus* (280–283), Reaction 58 is the major route for carbon dioxide fixation. In most bacteria, owever, and in animal and some plant tissues, Reaction 57 is reserve counts for the uptake of carbon dioxide (284, 285).

The nucleotide in Reaction 57 was first thought to be ATP (284, 5), but was later shown to be ITP (286). The effectiveness of ATP earlier experiments (273, 286–289) could be attributed to its conmination by ITP and to the presence of transphosphorylating enmes (52, 290) in the oxaloacetate carboxylase preparation. An absolute requirement for ITP was found with an enzyme obtained from mb liver (291), whereas an enzyme purified from avian liver had a gher activity with GTP than with ITP (292). In contrast, a purified east enzyme utilizes only ATP (292a). It has been suggested 293, 294) that a phosphorylated derivative of oxaloacetate may be rmed in Reaction 57, but conclusive evidence on this point is still cking (295).

The biosynthesis of PEP may also be achieved via the *pyruvate osphokinase* (296–298) reaction (cf. Eq. 54). Studies on the synthesis pyruvate from PEP showed that ADP can be replaced by IDP d GDP (299). A comparison of Eqs. 54 and 57 reveals that EP may participate with a nucleoside diphosphate in two similar retions, both yielding a nucleoside triphosphate. Thus, an unequivocal monstration (287, 300) of oxaloacetate carboxylase can be attained ly after the preparation has been freed from pyruvate phosphokinase. n alternate method of following Reaction 57 makes use of the nucleole-dependent exchange of $C^{14}O_2$ into oxaloacetate.

The equilibrium position of Reaction 54 lies far to the left, thus, ly small amounts of PEP can be formed by the action of pyruvate osphokinase, unless the products are removed by coupled reactions. owever, the synthesis of PEP from pyruvate can easily take place by e following sequence of reactions (301): (*a*) reductive carboxylation pyruvate to malate by the "malic enzyme" (300); (*b*) oxidation of alate to oxaloacetate; and (*c*) decarboxylation of oxaloacetate via eaction 57.

c. Function. The transphosphorylation of nucleotides from PEP via eaction 54 constitutes one of the key reactions in glycolysis. The uilibrium of Reaction 54 favors the formation of the triphosphate d provides one of the two net "energy-rich" bonds generated in

glycolysis. Phosphate may also be transferred from PEP to glycerol and other alcohols by the action of phosphatase (*299*); the importance of this reaction in cellular economy is probably limited.

The formation of oxaloacetate from PEP by reversal of Reaction 57 provides an important mechanism for the fixation of carbon dioxide by a variety of animal, plant, and microbial cells. In heterotrophs, Reaction 57 is the chief mechanism for carbon dioxide fixation (*279, 302*). In plants and many autotrophic bacteria (*91i–91k, 303–306a*), the carboxydismutase reaction (discussed in Vol. I, Chapter 9) appears to be the major route of entry of carbon dioxide. However, these systems also contain oxaloacetate carboxylase (*303, 307–310*), although this enzyme may function solely to provide oxaloacetate for the synthesis of citrate or amino acids.

In autotrophs, carbon dioxide fixation is also achieved (*293, 301, 311–313*) via Reaction 59:

$$\text{PEP} + \text{CO}_2 \rightarrow \text{Oxaloacetate} + \text{P}_i \qquad (59)$$

This irreversible reaction is catalyzed by *PEP carboxylase*, and does not require the participation of any nucleotide. The enzyme has a high affinity for carbon dioxide (*311, 313*), thereby permitting Reaction 59 to take place at low tensions of carbon dioxide. It has been suggested that the action of PEP carboxylase would account for the accumulation of C_4 acids by plants in the dark (*301, 311–313*), and for the fixation of carbon dioxide by *Thiobacillus thio-oxidans* (*308–309a*). The latter autotroph is thus able to utilize carbon dioxide as the sole carbon source in an acid environment, in spite of the fact that the carbon dioxide concentration is extremely small.

It has been proposed that oxaloacetate carboxylase may play an important role in glycogenesis through the synthesis of PEP (*314*). Oxaloacetate could be formed from the di- and tricarboxylic acids of the citric acid cycle (*315–317*), or could arise by reductive carboxylation of pyruvate by the "malic enzyme," followed by oxidation of malate to oxaloacetate. "Malic enzyme," malic dehydrogenase, and oxaloacetate carboxylase have been found in tissues (*284, 287, 288, 301*) that are unable to produce appreciable quantities of PEP by Reaction 54.

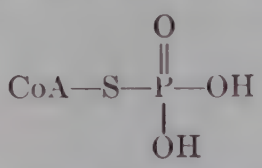
It has been postulated also that PEP condenses with erythrose-4-phosphate to yield 2-keto-3-deoxy-7-phosphoglucoheptonic acid (*318, 319*), which, in turn, may be dephosphorylated and cyclized to 5-dehydroshikimic acid (*320*). The latter compound is an intermediate in the synthesis of tyrosine, tryptophan, and other aromatic compounds. The production of 2-keto-3-deoxy-8-phosphooctonic acid (a precursor of certain cell wall constituents) from PEP and arabinose-5-phosphate has been demonstrated with a bacterial system (*320a*).

Other enol phosphates have been considered as possible intermediates in enzymatic reactions: vinyl phosphate in photosynthesis (*320b*), and phenol phosphates in oxidative phosphorylation (*320c–320e*).

d. Distribution. It is expected that PEP would be found in all plant, animal, and microbial tissues capable of glycolysis (*277*). In addition, specific enzymes (cf. Eqs. 54, 57, 59) capable of producing or utilizing PEP have been reported from a wide variety of sources: spinach leaves (*293, 311*), Crassulean plants (*312, 313*), wheat germ (*295, 301*), algae (*303*), yeast (*296, 321*), photosynthetic (*307*), autotrophic (*306, 308–310*), and heterotrophic (*279, 302, 318–320*) bacteria, and mammalian tissues (*273, 287, 288, 291–299, 314–317*).

5. *Thiol Phosphate: S-Phosphoryl Coenzyme A*

Evidence has been presented (*49*) that S-phosphoryl coenzyme A is formed as an intermediate in the activation of succinate by *Escherichia coli.* This compound has the general structure



The formula of the CoA molecule is detailed in Fig. 11,A. The over-all reaction for the activation of succinate is shown in Eq. 60.

$$\text{Succinate} + \text{ATP} + \text{CoA} \rightleftharpoons \text{Succinyl-CoA} + \text{ADP} + \text{P}_i \qquad (60)$$

This multicomponent reaction has been separated into two steps: (*a*) an interaction of ATP and CoA mediated by the enzyme, *Coenzyme A thiokinase;* and (*b*) a transfer reaction, catalyzed by the enzyme, *phosphoryl-CoA transferase.* These reactions are indicated in Eqs. 61 and 62.

$$\text{ATP} + \text{CoA} \rightleftharpoons \textit{S}\text{-Phosphoryl-CoA} + \text{ADP} \qquad (61)$$

$$\textit{S}\text{-Phosphoryl-CoA} + \text{Succinate} \rightleftharpoons \text{Succinyl-CoA} + \text{P}_i \qquad (62)$$

The enzymes catalyzing Reactions 61 and 62 have been partially purified with respect to each other, thus permitting the isolation (*49, 49a*) of S-phosphoryl-CoA via Reaction 61. Equilibrium studies of this reaction indicate that the ΔF of hydrolysis of S-phosphoryl-CoA is approximately 2 kcal./mole more negative than that of ATP (*49*).

It has not been determined whether the postulated intermediate, phosphoryl-CoA, is formed by other bacteria or by plant and animal tissues. Data obtained in kinetic studies (*169*) with the succinate-activating enzyme of muscle and spinach were not compatible with the formation of phosphoryl-CoA. However, preparations from brain have

been reported (*321a*) to carry out Reaction 61; reversal of this reaction using chemically synthesized phosphoryl-CoA was also indicated.

6. *Amidine Phosphates: Creatine, Arginine, and Other Guanidophosphates*

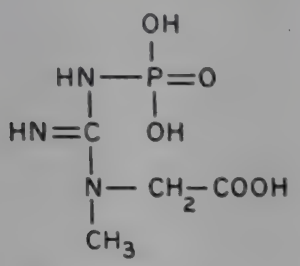
a. Preparation and Identification. A labile *N*-phosphate compound was first discovered by Eggleton and Eggleton (*322, 323*) in mammalian muscle, and was subsequently isolated and identified as phosphocreatine by Fiske and SubbaRow (*324, 325*). Phosphoarginine was isolated and identified from extracts of invertebrate muscle by Meyerhof and Lohmann (*326*). Three additional "energy-rich" *N*-phosphate compounds have been encountered more recently (*327–330*): i.e., phosphoguanidoacetate, phosphoguanidotaurine, and phosphoguanidoethylserylphosphate ("lombricine"). The structures of these latter compounds were elucidated after isolation in crystalline form from tissues of certain species of Annelida, Sipunculoidea, and Nemertea (*105, 327–333*). None of the above amidine phosphates is found in plants (*334*) or in bacteria (*335*). Formulas of the 5 compounds, often termed "phosphagens," are given in Fig. 10.

Phosphocreatine, phosphoguanidoacetate, and phosphoguanidotaurine may be synthesized by phosphorylation with $POCl_3$ of creatine (*336, 337*), guanidoacetate (*331, 338, 339*), and guanidotaurine (*331, 338, 339*). Phosphoarginine has not yet been synthesized, but can be isolated from the muscle of the crayfish *Jasus verreauxi* (*340, 340a*). Phosphoguanidoethylserylphosphate has been synthesized recently (*340b*), following the interesting discovery that the serine moiety has the D-configuration (*340c*).

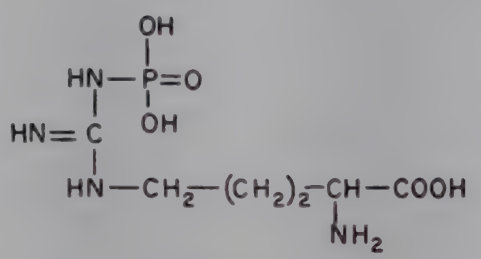
A detailed discussion of the methods for identifying these compounds is presented in the comprehensive review on amidine phosphates by Ennor and Morrison (*341*). In much of the early work, identification of phosphocreatine (*342, 343*) and phosphoarginine (*344*) was based on the lability of the phosphate bond in acid. Phosphocreatine was estimated directly by the Fiske-SubbaRow method for P_i, after an initial precipitation of P_i with Ca^{2+}; phosphoarginine required more prolonged hydrolysis in acid. In tissues containing both phosphoarginine and phosphocreatine, the two compounds were distinguished by differences in rates of hydrolysis in acid molybdate (*343*). These procedures have been criticized (*345*) because of the difficulty in quantitatively removing P_i by precipitation with Ca^{2+}, and because labile phosphate compounds other than phosphocreatine and phosphoarginine may be hydrolyzed under the conditions of testing.

More recently, free creatine resulting from acid hydrolysis of phos-

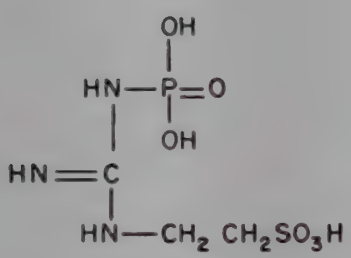
phocreatine, has been determined colorimetrically (*345*), or by reaction with creatine phosphokinase (*345a*). Phosphocreatine can also be converted to creatinine by a reaction with acid molybdate (*346*). Separation of phosphate esters chemically (*336, 347*) or by passage through an ion exchange resin (*348, 349*) has been used to provide sufficient



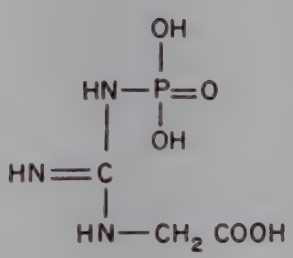
Phosphocreatine



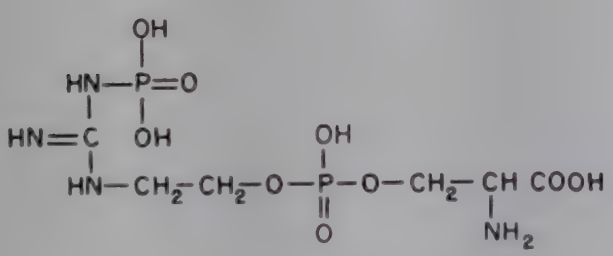
Phosphoarginine



Phosphoguanidotaurine



Phosphoguanidoacetate



Phosphoguanidoethylserylphosphate

FIG. 10. Amidine phosphates.

concentration of phosphocreatine to permit determination of the phosphate:creatine ratio. Both phosphocreatine and creatine can be identified by means of paper chromatography (*336, 340, 349, 350*).

Concentration of phosphoarginine by chemical methods (*351*) or by ion exchange resins (*341*) has also been successful and fractions obtained by these methods have been analyzed (*351*) for the phosphate: arginine ratio. Arginine can be determined colorimetrically by the Weber modification (*352*) of the Sakaguchi method, or by reaction

with purified arginine phosphokinase (*340, 350, 353*) or arginase (*340*). A simple colorimetric assay for arginine after hydrolysis of tissue extracts has also been widely used as a measure of phosphoarginine (*343, 348, 354*). Phosphoarginine or free arginine may be separated and identified by paper chromatography (*347–348a, 354*).

Studies of the distribution of phosphoguanidoacetate, phosphoguanidotaurine, and phosphoguanidoethylserylphosphate in animal tissues have depended on preliminary fractionation of extracts followed by paper chromatography (*105, 328–333, 341, 349*). The nonphosphorylated guanidine compounds can also be separated chromatographically (*355*) or identified by reaction with the specific phosphokinases (*333, 338, 356*). After prolonged acid hydrolysis of phosphoguanidoethylserylphosphate, guanidoethylserylphosphate, guanidoethylphosphate, guanidoethanol, phosphate, and serine can be detected by paper chromatography (*105*).

b. Biosynthesis. Phosphocreatine and phosphoarginine are formed by the ATP-dependent phosphorylation of creatine and arginine as shown in Reactions 63 and 64:

$$\text{Creatine} + \text{ATP} \rightleftharpoons \text{Phosphocreatine} + \text{ADP} \qquad (63)^*$$

$$\text{Arginine} + \text{ATP} \rightleftharpoons \text{Phosphoarginine} + \text{ADP} \qquad (64)$$

The distribution of the specific kinases mediating these reactions is correlated with the accumulation of the specific *N*-phosphate compound in a given tissue (*342, 357*). The properties of creatine phosphokinase have been described in considerable detail (*345a, 358–361*). The enzyme has been crystallized (*362, 363*), and studies have been made of the kinetics of the reaction (*46, 364, 365*). Arginine phosphokinase has also been purified and its properties, especially the interactions with metal ions (*365a*), have been determined (*353, 366*). Crystallization of the enzyme has been reported (*367, 367a*).

Invertebrates whose tissues do not contain phosphocreatine either (*a*) do not possess the enzymes necessary for methylation of guanidoacetate to creatine, (*b*) do not contain guanidoacetate, or (*c*) lack both methylation enzymes and methyl acceptor (*368, 369*). Conversely, invertebrate tissues having phosphocreatine also contain free guanidoacetate and are able to methylate this compound at the expense of either active methionine, betaine, or choline (*369*).

Studies have been made of the properties and kinetics of phosphokinases (*338, 356, 365a, 366, 370*) functioning in the synthesis of phos-

* The direct phosphorylation of creatine by 1,3-diphosphoglycerate has been reported recently (*356a*).

phoguanidoacetate and phosphoguanidotaurine, as shown in Eqs. 65 and 66:

$$\text{Guanidoacetate} + \text{ATP} \rightleftharpoons \text{Phosphoguanidoacetate} + \text{ADP} \quad (65)$$

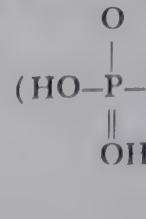
$$\text{Guanidotaurine} + \text{ATP} \rightleftharpoons \text{Phosphoguanidotaurine} + \text{ADP} \quad 66)$$

It has been assumed that guanidoethylserylphosphate is phosphorylated at the expense of ATP by reactions similar to Eq. 65 and 66 but the responsible enzyme has not yet been demonstrated. The mechanism of formation of guanidoethylserylphosphate has not been investigated.

c. Function. The recognition (*324–326*) that phosphocreatine and phosphoarginine play an important role in an energy-requiring reaction was achieved in the late 1920's. As described more fully in Vol. II, Chapter 5, these compounds regenerate the ATP needed for muscle contraction, and are, in turn, resynthesized by ATP when sufficient amounts of the latter substance accumulate; Reactions 63 and 64 are completely reversible. Since Reactions 65 and 66 are also reversible, and phosphoguanidoacetate and phosphoguanidotaurine are the only phosphogens found in some animals, it is assumed that these compounds can also be mobilized for muscle contraction in a manner fully analogous to phosphocreatine and phosphoarginine. As stated above, no enzyme capable of phosphorylating guanidoethylserylphosphate has yet been demonstrated. However, this compound is the only phosphagen found in the muscle tissues of *Lumbricus terrestris* and the amount present (*105*) is comparable to that of *N*-phosphate compounds in vertebrate tissues. On the basis of these considerations, the not unreasonable assumption has been made (*105, 327, 329*) that this compound, too, functions in ATP regeneration.

It is obvious that the ATP formed by transphosphorylation from the phosphagens can be utilized for synthetic reactions,* or for mechanical work, ion transport, or nerve transmission (*371*). While the greatest amounts of amidine phosphates are found in muscle, these compounds may occur in other tissues as well. For example, considerable amounts of phosphocreatine have been found in mammalian liver (*341*) and, similarly, varying amounts of phosphoarginine have been reported from different invertebrate tissues (*333*).

* It has been shown that, in the presence of a phosphatase, phosphocreatine can directly phosphorylate glucose and glycerol without intervention of the adenylic system (*370a, 370b*). Likewise, a bacterial system has been found which can utilize, for the phosphorylation of hexoses, phosphoramide (*370c*):



d. Distribution. Interest in the distribution of the *N*-phosphate compounds in various species of animals stems from attempts to correlate the presence of phosphocreatine and phosphoarginine in animal phyla with certain phylogenetic relationships (*342*, *357*, *371*, *372*). These relationships (for example, of the echinoderms to hemichordates and to vertebrates) were originally postulated on the basis of morphological and embryological comparisons (*372*, *373*) and appeared, at first, to receive support from the pattern of distribution of the "energy-rich" amidine phosphates.

As shown in Table II, an early survey of animals of different phyla showed that neither phosphoarginine nor phosphocreatine was found in Protozoa, Porifera, Annelida, and Sipunculoidea. Phosphoarginine, but

TABLE II

DISTRIBUTION OF PHOSPHOARGININE AND PHOSPHOCREATINE IN ANIMALS[a]

Phylum	Phosphoarginine	Phosphocreatine
Protozoa, Porifera, Annelida, Sipunculoidea	−	−
Coelenterata, Platyhelminthes, Nemertea, Mollusca, Phoronida, Arthropoda	+	−
Echinodermata		
Crinoidea, Asteroidea, Holothuroidea	+	−
Ophiuroidea	−	+
Echinoidea	+	+
Hemichordata	+	+
Chordata		
Urochordata	+	−
Cephalochordata	−	+
Vertebrata	−	+

[a] From Baldwin (*14*, *371*).

not phosphocreatine, was found in Coelenterata, Platyhelminthes, Nemertea, Mollusca, Arthropoda, and in some members of the Echinodermata, Hemichordata, and Chordata. Phosphocreatine, on the other hand, was found in all classes of the Vertebrata and also in certain of the echinoderms, hemichordates, and chordates. Phosphoarginine was not found in any of the vertebrate classes. The presence of both phosphocreatine and phosphoarginine in echinoids and hemichordates supported the view that these groups are related to each other and to the chordates. The presence of only phosphocreatine in the Cephalochordata was taken as an indication of a relationship of this group to the vertebrates (*342*, *371*, *372*).

As outlined in Table III (cf. also references *341* and *348*), later investigations disclosed many exceptions to the above generalization. Thus, certain species of Annelida were found to have both phosphoarginine and phosphocreatine. However, some closely related genera of annelids [for example *Neanthes* and *Nereis* (*343*)], and even congeneric species, differed with respect to the type of *N*-phosphate compound present in the muscle tissue. For example, *Glycera gigantea* was shown to have both phosphoarginine and phosphocreatine, whereas *G. convoluta* and *G. dibranchiata* had only phosphocreatine (*333*). Similar differences were found in the echinoderms, for example, between the related echinoids *Centrostephanus rodgersii* and *Heliocidaris erythrogramma* (*340*), and also in the chordates [cf. *Balanoglossus clavigerus* and *B. salmoneus* (*342–342b*)].

Further investigations disclosed that the tissues of a given animal may vary with regard to the kind of amidine phosphate present (*340, 348*). For example, phosphocreatine has been found in ripe sperm, but not in eggs, muscle, intestine, or immature sperm of the starfish *Marthasterias glacialis*. Similarly, sperm from the holothurian *Leptosynapta inhaerens*, the polychaete *Arenicola marina*, the sipunculid *Sipunculus nudus* and the coelenterate *Calliactis parasitica* contain phosphocreatine, whereas the muscle tissues do not (*348*). Phosphocreatine has also been found in the testicular tissues of *Strongylocentrotus dröbachiensis* (*351, 376*) and *Sphaerechinus granularis* (*348*), whereas the unfertilized eggs contain phosphoarginine (*348, 351*).

The muscle tissues of some worms have been found to lack both phosphocreatine and phosphoarginine, but have either phosphoguanidotaurine, phosphoguanidoacetate, or phosphoguanidoethylserylphosphate. The latter compound has been isolated only from muscle of the earthworm *Lumbricus terrestris*. Perhaps the examination of other species of oligochaetes will disclose that this compound has a wider distribution. Additional, and as yet unidentified, guanido compounds have also been found (*105, 332, 349*) and it is possible that phosphorylated derivatives of such compounds may function, too, as reservoirs of "energy-rich" phosphate.

It appears from the above discussion that the distribution of "energy-rich" *N*-phosphate compounds in a number of phyla is not constant and, in fact, varies between related genera, within a genus, and even between the organs of a single animal. Thus, newer and more extensive data on the distribution of *N*-phosphate compounds are not in accord with the accepted interphyletic relationships. In addition, some of the phyla examined also have phosphoguanidoacetate, phosphoguanidotaurine, and phosphoguanidoethylserylphosphate. The original generaliza-

TABLE III

DISTRIBUTION OF *N*-PHOSPHATE COMPOUNDS IN CERTAIN GROUPS OF ANIMALS[a]

Animal	Tissue	PA	PC	PG-Ac	PG-T	PG-ESP	References
Protozoa							
Tetrahymena geleii	Entire animal	+	−				*348*
Porifera							
Halichondria panicea	Entire animal	+	−				*348, 374, 374a*
Hymeniacidon caruncula	Entire animal	+	−				
Thetia lyncurium	Entire animal	−	+				
Coelenterata							
Actinia equina	Entire animal	+	−				*348*
Anemonia sulcata	Entire animal	+	t[b]				*348*
Calliactis parasitica	Muscle	+	−				*348*
Nematoda							
Ascaris lumbricoides	Entire animal	+	−				*348*
Nemertea							
Lineus gesserensis	Muscle	−	−	+	−		*330*
Sipunculoidea							
Phascolosoma elongatum	Muscle	t	−	−	+		*330, 348*
Sipunculus nudus	Muscle	−	−	−	+		*348*
	Eggs + sperm	+	+				
Annelida							
Polychaeta							
Arenicola marina	Muscle	−	−	−	+	−	*330–333, 366*
Myxicola infundibulum							
Clymene lumbricoides							
Lanice conchilega							
Sabellaria alveolata							
Nephtys caeca	Muscle	−	−	+	−	−	*330, 348, 366*
Nereis fucata							
Nereis diversicolor							
Eulalia viridis	Muscle	+	t				*333*
Hermione hystrix	Muscle	−	+				*333, 366*
Aphrodite aculeata							
Glycera convoluta	Muscle	−	+				*344, 348*
Glycera gigantea	Muscle	+	+				*344, 348*
Audouinia tentaculata	Muscle	t	+				*348*
Dasybranchus caducus	Muscle	t	+				*348*

[a] PA = phosphoarginine; PC = phosphocreatine; PG-Ac = phosphoguanidoacetic acid; PG-T = phosphoguanidotaurine; PG-ESP = phosphoguanidoethylserylphosphate.

[b] t = traces.

TABLE III (*Continued*)

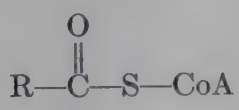
Animal	Tissue	PA	PC	PG-Ac	PG-T	PG-ESP	References
Polychaeta (*Cont.*)							
Halosydna gelatinosa	Muscle	+	+				*333*
Nephtys humbergii	Muscle	+	−				*333*
Oligochaeta							
Lumbricus terrestris	Muscle	−	−	−	−	+	*105, 327, 348, 349*
Echinodermata							
Asteroidea							
Marthasterias glacialis	Muscle	+	−				
	Eggs	+	−				*348*
	Sperm	−	+				
Asterias for besi	Sperm	−	+				*376*
	Eggs	+	−				*351*
Holothuroidea							
Leptosynapta inhaerens	Muscle	+	−				*348, 357, 375, 376*
Holothuria tubulosa	Sperm	−	+				
Ophiuroidea							
Amphipholis squamata	Entire animal	+	+				*348*
Ophiothrix fragilis							
Echinoidea							
Sphaerechinus granularis	Muscle	+	+				
Strongylocentrotus drōbachiensis	Eggs	+	−				*348, 351, 376*
	Sperm	−	+				
Heliocidaris erythrogramma	Muscle	+	+				*340*
Centrostephanus rodgersii	Muscle	+	−				*340*
Arbacia punctulata	Muscle	+	−				*343*
	Sperm	−	+				*376*
Strongylocentrotus purpuratus	Sperm	+	+				*342*
Paracentrotus lividus	Sperm	+	+				*357*
Chordata							
Urochordata							
Ascidia mentula	Entire animal	+	−				*342a*
Pyura stolonifera	Entire animal	−	+				*376a*
Enteropneusta							
Balanoglossus clavigerus	Entire animal	−	+				*340b*
B. salmoneus	Entire animal	+	+				*342, 342a*
Cephalochordata							
Amphioxus sp.	Entire animal	−	+				*376b*

tion that invertebrates characteristically have phosphoarginine while vertebrates have phosphocreatine now also requires modification since a number of phyla (Porifera, Sipunculoidea, Annelida, Echinodermata, Hemichordata, and Chordata) have been found to contain both compounds.

B. Derivatives of Carboxylic Acid

1. *Acyl Thioesters*

a. Preparation and Identification. Acyl thioesters, whose general structure is given by the type formula



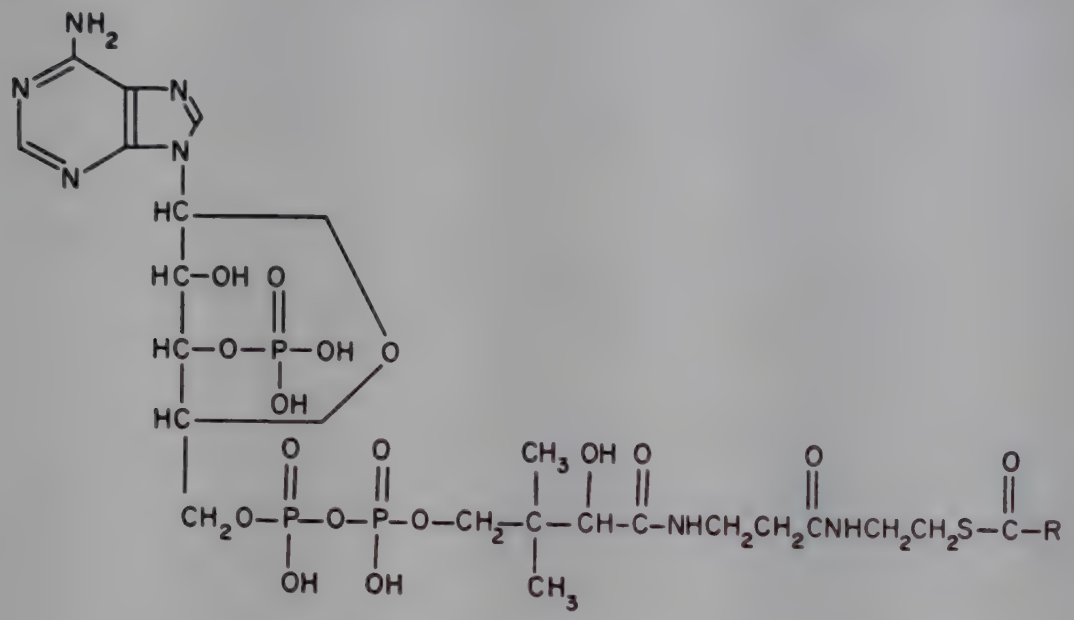
may be considered as quasi-anhydrides, since thiols ($pK = 7$–9) are stronger acids than alcohols ($pK = 13$–15). As anhydrides, these compounds are similar to acyl or enol phosphates, or to pyrophosphates, and it is not surprising, therefore, to find that they fall into the category of "energy-rich" materials. From the viewpoint of energy storage, the most important members of the acyl thioester group are the many acyl derivatives of Coenzyme A (Fig. 11,A), particularly acetyl-CoA and succinyl-CoA. Two additional thioesters, the acyl derivatives of glutathione (Fig. 11,B) and lipoic acid (Fig. 11,C), are quantitatively less important as energy storage forms, and will be discussed only briefly.

The first acyl thioester available in pure form was acetyl-CoA, originally called "active acetate." This compound was isolated from yeast by Lynen and co-workers (*377*) following its earlier implication as an intermediate in the acetylation of sulfanilamide (*378*) and choline (*379*). The subsequent discovery that CoA was involved as a transient carrier of many acyl groups in a wide variety of metabolic reactions (*380–382*) left little doubt as to the importance of this coenzyme in intermediary metabolism.

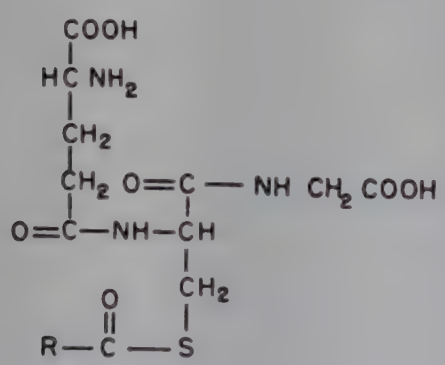
The total synthesis of CoA has been achieved (*383*, *383a*), and acyl derivatives of CoA are readily synthesized by several methods: (*a*) the reaction of an acid anhydride (*384*) or mixed anhydride (*385*) with the thiol; and (*b*) the ester exchange reaction (*386*). Enzymatic syntheses are also commonly employed, as for example, through the action of specific "activating enzymes" (*387*), phosphotransacetylase (*188*), or CoA transphorase (*388*) (described below).

Acyl thioesters react with hydroxylamine (*184*) at neutral pH to yield the corresponding acyl hydroxamates, which may be separated and identified by paper chromatography (*162*) or measured colori-

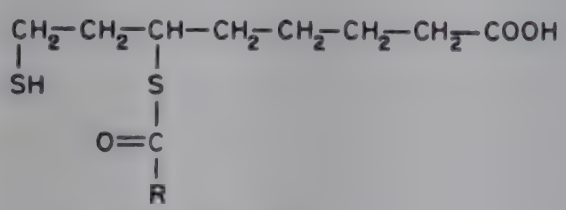
metrically. The acyl thioesters themselves may also be separated by paper chromatography (389), or they may be detected by virtue of their absorption band at approx. 230 mμ (*48*, 389). Specific enzymatic



A. Acyl CoA (R = acetyl, butyryl, succinyl, benzoyl, etc.)



B. Acyl Glutathione



C. Acyl lipoic acid

FIG. 11. Acyl thioesters.

assays have been devised for various acyl CoA derivatives; for example, the reaction with phosphotransacetylase and arsenate is used to measure acetyl CoA quantitatively (as discussed in Section III,A,3,*a* of this chapter). A nonspecific assay for acyl thioesters involves hydrolysis in

alkali, followed by an estimation of the liberated thiol group using the quantitative nitroprusside (*390*) or *p*-chloromercuribenzoate (*391*) method.

b. Biosynthesis. A number of acyl derivatives of CoA originate by two general enzymatic reactions: the direct activation of the organic acid (the mechanism of Reaction 67 has been discussed in Section III,A,3,*c* of this chapter), and the transphorase reaction (Eq. 68).

$$\text{RCOOH} + \text{ATP} + \text{CoA} \rightleftharpoons \text{R}-\overset{\overset{\text{O}}{\|}}{\text{C}}-\text{S}-\text{CoA} + \text{AMP} + \text{PP} \qquad (67)$$

$$\text{Acetyl-CoA} + \text{RCOOH} \rightleftharpoons \text{Acetate} + \text{R}-\overset{\overset{\text{O}}{\|}}{\text{C}}-\text{S}-\text{CoA} \qquad (68)$$

Acetyl-CoA may be formed via Reactions 67 and 68 and also by a number of other reactions. The following examples may be cited: the CoA-dependent cleavage of acetoacetyl-CoA (*392–396*), β-ketoadipyl-CoA (*397*), α-methyl acetoacetyl-CoA (*398*), and malonyl-CoA (*398a,b*); the oxidation of acetaldehyde (*196, 399*); the cleavage of citrate to oxaloacetate and acetyl-CoA (*400*); transacetylation from acetyl phosphate (*187*); and oxidative decarboxylation of pyruvate (*401*), and malonic semialdehyde (*401a*).

Succinyl-CoA also arises via Reactions 67 and 68. In animal tissues (*393, 394*), a specific transferase is required for the transfer of CoA from acetoacetyl-CoA to succinate; in bacteria (*163*), CoA is transferred to succinate from acetyl-CoA or propionyl-CoA. Synthesis of succinyl-CoA may also occur by the CoA-dependent cleavage of β-ketoadipyl CoA (*397*), by carboxylation of propionyl-CoA in bacteria (*163*), by isomerization of methylmalonyl-CoA in animal tissues (*402*), and by oxidative decarboxylation of α-ketoglutarate (*401, 403*).

Specific enzymes are known for the activation of benzoate, propionate, butyrate, and other acids according to Eq. 67, and formate, propionate, butyrate, lactate, and other acids interact with acetyl-CoA in Reaction 68 (*404*). Many additional CoA derivatives are synthesized by specific reactions which will not be detailed here: for example, various intermediates during the sequence of fatty acid oxidation or during the degradation of valine, leucine, and isoleucine.

Acyl derivatives of glutathione may be formed by several *thiol transacetylases* (*405*), as shown in Reaction 69:

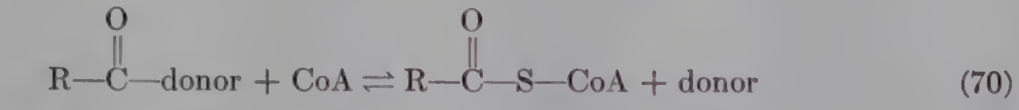
$$\text{Acyl-CoA} + \text{RSH} \rightleftharpoons \text{Acyl}-\text{SR} + \text{CoA} \qquad (69)$$

An acyl glutathione is also synthesized as an intermediate in the isomerization of methylglyoxal to lactate (*197, 406*), and has been postu-

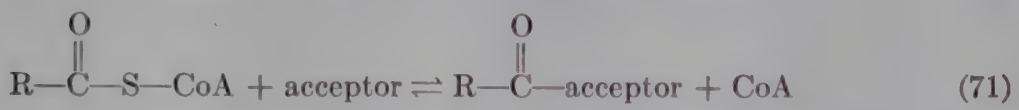
lated as an enzyme-bound intermediate in the oxidation of 3-phosphoglyceraldehyde (discussed in section III,A,3,*b* of this chapter).

In bacterial systems (*401*), acyl lipoates have been identified as intermediates in the oxidative decarboxylation of α-ketoglutarate, α-ketobutyrate, and pyruvate. Lipoic acid is the acceptor for the acyl group in the above reactions, and the acyl group is subsequently transferred to CoA by a reaction similar to that shown in Eq. 69.

c. Function. Acyl thioesters represent an "activated form" for the acyl group in a variety of metabolic reactions. As with other coenzymes, CoA may be considered as a reversible carrier for a mobile metabolic group (*407*), in this instance the acyl moiety. The transfer of the acyl group may be written in two steps:



$$R{-}\overset{\overset{O}{\|}}{C}{-}\text{donor} + \text{CoA} \rightleftharpoons R{-}\overset{\overset{O}{\|}}{C}{-}S{-}\text{CoA} + \text{donor} \qquad (70)$$

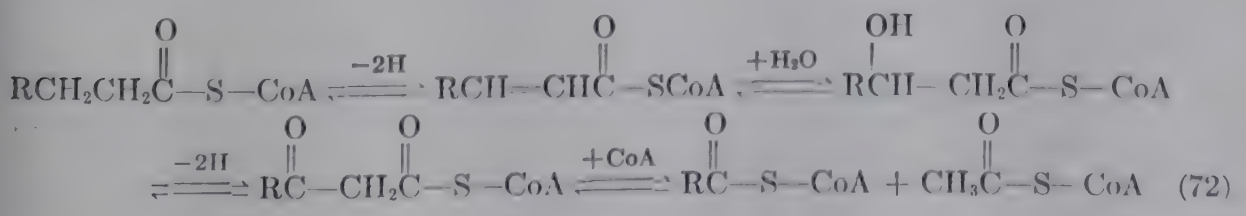


$$R{-}\overset{\overset{O}{\|}}{C}{-}S{-}\text{CoA} + \text{acceptor} \rightleftharpoons R{-}\overset{\overset{O}{\|}}{C}{-}\text{acceptor} + \text{CoA} \qquad (71)$$

This formulation accounts for the many reactions where acetate, acetyl phosphate, citrate, acetoacetate, β-ketoadipate, and α-methyl acetoacetate serve as acetyl donors, while oxaloacetate (to form citrate), formate (to form pyruvate), glucosamine (to form *N*-acetyl glucosamine), amino acids (to form *N*-acetyl amino acids), choline (to form acetyl choline), sulfanilamide and aromatic amines (to form the *N*-acetyl amines), or acetyl-CoA (to form acetoacetyl-CoA) serve as acetyl acceptors.

Similarly, α-ketoglutarate and succinate may act as succinyl donors. Glycine (to form α-amino-β-ketoadipic acid) and acetyl-CoA (to form β-ketoadipic acid) are succinyl acceptors. Succinyl-CoA may also function in the formation of propionate by bacteria and in the oxidation of propionate by mammalian tissues. The function of the other principal acyl thioesters, namely acyl derivatives of glutathione and lipoic acid, is also that of acyl transfer.

In fatty acid oxidation, reviewed in detail elsewhere (*377, 408, 409*), the intermediate acyl-CoA complex is maintained during a series of reactions involving a structural change in the acyl moiety, as shown in Reaction 72.



$$\begin{aligned} &RCH_2CH_2\overset{\overset{O}{\|}}{C}{-}S{-}\text{CoA} \xrightleftharpoons{-2H} RCH{=}CH\overset{\overset{O}{\|}}{C}{-}S\text{CoA} \xrightleftharpoons{+H_2O} RCH(OH){-}CH_2\overset{\overset{O}{\|}}{C}{-}S{-}\text{CoA} \\ &\xrightleftharpoons{-2H} RC(=O){-}CH_2\overset{\overset{O}{\|}}{C}{-}S{-}\text{CoA} \xrightleftharpoons{+\text{CoA}} RC(=O){-}S{-}\text{CoA} + CH_3\overset{\overset{O}{\|}}{C}{-}S{-}\text{CoA} \end{aligned} \qquad (72)$$

From the standpoint of energetics, acyl thioesters are essentially isoenergetic with ATP (cf. Table I), and there is little doubt that, besides the specific metabolic functions indicated above, these compounds play a major role in energy storage and utilization.

d. Distribution. Except for acetyl-CoA, acyl-CoA derivatives have not been isolated, as such, from tissues, and it is probable that their steady-state level is rather low. However, the existence of many enzymes that produce or utilize acyl derivatives of CoA suggests that these intermediates actually are present in a wide variety of tissues and cells. Perhaps more significant is the fact that the vitamin component of CoA, pantothenic acid, is ubiquitously distributed in nature (*410*). The level of CoA in tissues, as estimated by the sulfanilamide-acetylating enzyme systems, is given in a review by Novelli (*411*).

2. *Acetyl Imidazole*

Acetyl imidazole, represented by Fig. 12, is a recently discovered

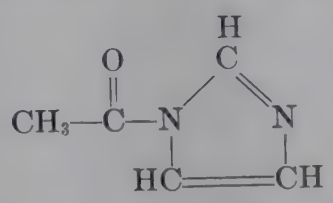


FIG. 12. Acetyl imidazole.

(*48*, *412*) addition to the class of "energy-rich" compounds. The compound is synthesized from acetyl-CoA by the action of the enzyme, *imidazole acetylase*, as shown in Reaction 73 or from acetyl phosphate by a combination of Eqs. 74 and 73.

$$\text{Acetyl-CoA} + \text{Imidazole} \rightleftharpoons \text{Acetyl imidazole} + \text{CoA} \quad (73)$$

$$\text{Acetyl phosphate} + \text{CoA} \rightleftharpoons \text{Acetyl-CoA} + P_i \quad (74)$$

Reaction 73 may be followed spectrophotometrically since acetyl imidazole has its absorption maximum at a slightly longer wavelength (245 mμ) than acetyl-CoA (230 mμ). Kinetic studies (*48*) have shown that the ΔF of hydrolysis of acetyl imidazole is 2.8 kcal./mole more negative than that of acetyl phosphate. Acyl derivatives of imidazole have been prepared by chemical synthesis (*413*, *414*), as has the interesting compound, phosphoryl imidazole (*415*).

Acyl imidazoles can react with thiols, P_i, arsenate, sugars, and water (*47*, *48*, *415a*), and can serve as intermediates in the transfer of acyl groups from acyl adenylates to the above acceptors.

Imidazole brings about the nonenzymatic hydrolysis of esters (*416*, *417*). Likewise, an imidazole moiety may be involved in the chymotrypsin-catalyzed hydrolysis of esters, and it has been postulated that

an imidazole residue is located at, or near, the active site of this enzyme (*418–420*). The possible role of imidazole in phosphate transfer reactions has also been discussed (*415*).

To date, the net synthesis of acetyl imidazole has been demonstrated only with an enzyme preparation from *Clostridium kluyveri*. It is of interest that experiments with imidazole-containing compounds have shown that only urocanate (*48*) can replace imidazole in Reaction 73. From these considerations, it would appear that acyl imidazoles, like acyl adenylates, probably function as intermediate carriers of the "energy-rich" linkage in enzymatic reactions, but that they do not serve as major energy reservoirs in the cell.

C. Summary: Distribution of "Energy-Rich" Compounds

The distribution of "energy-rich" compounds in microbial, plant, and animal cells is so nearly universal that, with the exception of viruses (*421*) and perhaps rickettsia, possession of these compounds can be considered a basic attribute of all forms of life. Since the major biological processes (e.g., replication, growth, differentiation, maintenance, and aging) involve changes in energy, it is to be expected that cells should contain compounds capable of acting as energy reservoirs and enzyme systems for the utilization of these compounds. The "energy-rich" compounds may be categorized into: (*a*) energy reservoirs; (*b*) primary phosphorylating agents; and (*c*) transient intermediates in phosphorylating reactions, as shown in Fig. 13. Certain of the compounds which may serve to generate ATP (PEP and glyceryl phosphate) appear to be universally distributed, as is ATP, the primary phosphorylating agent. Other compounds which act as storage forms (inorganic polyphosphates, carboxyl phosphates, and amidine phosphates) appear to have a specialized distribution, while acyl thioesters, acyl adenylates, S-phosphoryl-CoA and acyl imidazoles are probably formed by most cells as intermediates in ATP-dependent reactions.

The relationships shown in Fig. 13 are consistent with the fact that the ΔF° of hydrolysis for ATP is *lower* than that of most other "energy-rich" compounds (cf. Table I). It follows from equilibrium considerations alone that there would be little tendency for ATP to interact with the precursor of another "energy-rich" compound, unless this endergonic reaction could be coupled with a second reaction, exergonic in nature. Thus, the energy of the system will tend to remain in the primary storage compound, ATP, until a specific demand for another "energy-rich" compound makes possible the endergonic transfer.

The universal distribution of ATP is illustrated by its isolation from the following diverse sources: higher plants (*422*, *423*) algae (*303*),

fungi (*424*), yeast (*99, 100*), numerous microorganisms (*50, 101*), protozoa (*425, 426*), arthropods (*103, 104, 427, 428*), annelids (*105*), echinoderms (*102, 429*), and all classes of vertebrates (*62, 93–96, 106–108*). The presence of ATP in cells from which it has not been specifically isolated may be inferred from the ability of these cells to carry out reactions which either yield or utilize ATP. For example, the existence of "energy-rich" *N*-phosphate compounds in certain cells (Tables II and III) implies the concomitant existence of ATP since the *N*-phosphate compounds are only known to be synthesized or utilized by transphosphorylation with ATP.

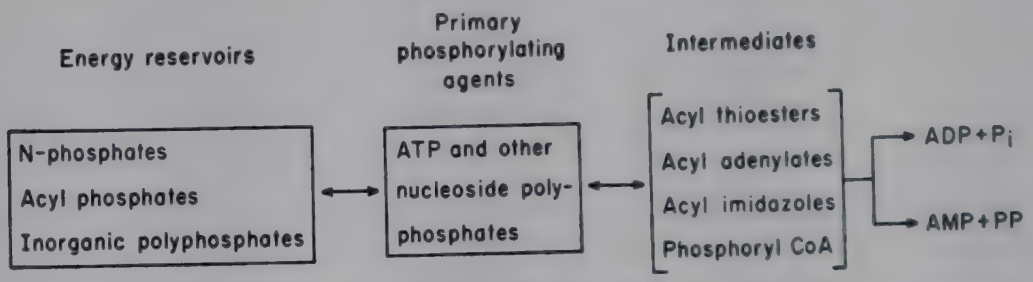


FIG. 13. Functional interrelationships of "energy-rich" compounds.

Since "energy-rich" compounds are required for major cellular processes, and are almost universally distributed, one may justifiably conclude that such compounds must have been extant relatively early in evolution.* However, an initial chemical synthesis of "energy-rich" compounds utilizing *soluble* phosphates would have been difficult because *insoluble* phosphate salts predominated at the time of the evolution of organic compounds (*430*). It has been suggested (*430–433*) that the environment during this period was anaerobic, thus favoring the creation of more reduced forms of phosphorus (i.e., phosphite and hypophosphite) which have greater solubility. Under these conditions, the synthesis of a typical "energy-rich" compound, phosphoguanidine, could have occurred by the condensation of carbon monoxide and ammonia to produce cyanamide, followed by reaction with ammonium phosphite to yield the precursor of phosphoguanidine (*430*). Formation of the more complex "energy-rich" molecules, such as ATP, would require the prior synthesis of purine, ribose, etc., and possibly the participation of a rudimentary matrix, such as clay particles (*432, 433*). Subsequent transphosphorylations could occur in the presence of divalent metal ions, such as Mg^{2+} or Mn^{2+} (*433a,b*).

Since the concentration of soluble phosphorus compounds in the early environment was small, the evolution of mechanisms for accumu-

* The initial appearance of ATP may well have been the decisive event in the transition from the inanimate to the animate state (*429a*).

lating this element in the form of readily available "energy-rich" storage compounds would have had great selective advantages. Whereas many of the "energy-rich" compounds are found in all forms of life, there appears to have been a fundamental dichotomy in the evolution of plants and animals with respect to storage forms of these compounds. Higher plants, algae, fungi, and bacteria contain polyphosphates and certain carboxyl phosphates which may serve as storage compounds: in animals, this function is performed by the amidine phosphates.

High and low molecular weight inorganic polyphosphates interact readily with ATP, as discussed earlier. The distribution and even the chemical characterization of polyphosphates in plants and bacteria have received little attention and, in fact, the precise role of this class of compounds in cellular economy is still imperfectly understood. On the other hand, carboxyl phosphates as energy storage compounds are apparently restricted to bacteria. It is to be hoped that future investigations will supply more detailed information on the distribution of individual polyphosphates or carboxyl phosphates within the classes of plants and microorganisms. Such data are presently available only for the amidine phosphates.

The distribution in animals of the "energy-rich" *N*-phosphate compounds at first appeared to be unambiguous: phosphoarginine was characteristic of invertebrates, while phosphocreatine was characteristic of vertebrates. This led, in fact, to the proposal that the animal kingdom be divided into the "creatinate" and "acreatinate" groups (*434*). A few invertebrates were found to have both phosphocreatine and phosphoarginine; these were considered, therefore, to be related to each other and to vertebrates. More detailed examination of greater numbers and varieties of animals and the discovery of further *N*-phosphate compounds revealed that the pattern of distribution of amidine phosphates is complex. More than one *N*-phosphate compound has been found in the following: Porifera, Coelenterata, Annelida, Sipunculoidea, Echinodermata, Hemichordata, and Chordata. Morphological and embryological comparisons show that although some phyla (Echinodermata, Hemichordata, and Chordata) having both phosphoarginine and phosphocreatine are related to each other; other phyla (for example, Annelida and Porifera), also possessing both compounds, are not closely related. In many phyla, the distribution of phosphoarginine and phosphocreatine may differ between closely related genera, between species, and even between tissues of a single animal. A number of animals contain additional *N*-phosphate compounds whose distribution cannot be correlated, at the present time, with an accepted evolutionary sequence. Thus, it may be concluded that the type of *N*-phos-

phate compound present in a given phylum does not appear to constitute a valid criterion of phyletic relationship.

It has been suggested that the distribution of *N*-phosphate compounds could be the result of convergent evolution, with the various amidine phosphates arising independently at different times in different animals. The distribution of these compounds could also be accounted for by postulating the evolution of a common synthetic pathway yielding the guanido group with subsequent mutations leading to the formation of two main compounds (phosphocreatine and phosphoarginine); the presence of both compounds in some invertebrates could be attributed to still later mutations (*435*). Wald (*436*) has suggested that if possession of either phosphocreatine or phosphoarginine were important for survival, selection would have eliminated the other. Thus, the wide distribution of phosphocreatine and phosphoarginine in the animal kingdom may indicate that they are nearly equivalent in function. The restricted distribution of phosphoguanidoacetate, phosphoguanidotaurine, and phosphoguanidoethylserylphosphate, on the other hand, may result from selection or rare mutations.

The above discussion has focused attention upon two of the current problems in this particular area of comparative biochemistry: namely, that (*a*) inadequate data on the distribution of certain classes of "energy-rich" compounds, notably inorganic polyphosphates and carboxyl phosphates, precludes any detailed phyletic correlation, while (*b*) the accumulation of more complete data on the amidine phosphates has illustrated the complexity of such relationships. Differences in the distribution of certain key biochemical compounds must obviously underlie morphological differences, and conversely, the morphological and embryological similarities of related organisms must result from the expression of common biochemical attributes. The identification of these key compounds, elucidation of their role in metabolism, and the analysis of their distribution in the plant and animal kingdoms will undoubtedly remain a major goal in the field of comparative biochemistry.

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References

1. N. W. Pirie, *in* "Perspectives in Biochemistry" (J. Needham and D. E. Green, eds.), p. 11. Cambridge University Press, London and New York, 1937.
2. H. J. Muller, *Science* **121**, 1 (1955).
3. A. Szent-Györgyi, *Science* **124**, 873 (1956).
4. B. W. Gabrio, C. A. Finch, and F. M. Huennekens, *Blood* **11**, 103 (1956).

5. J. von Liebig, *Ann.* **62,** 257 (1847).
6. M. E. Jones, *Yale J. Biol. and Med.* **26,** 80 (1953).
7. F. Lipmann, *Advances in Enzymol.* **1,** 99 (1941).
8. H. M. Kalckar, *Chem. Revs.* **28,** 71 (1941).
9. H. M. Kalckar, *Nature* **160,** 143 (1947).
10. H. M. Kalckar, *in* "Currents in Biochemical Research" (D. E. Green, ed.), p. 229. Interscience, New York, 1946.
11. P. Oesper, *in* "Phosphorus Metabolism" (W. D. McElroy and B. Glass, eds.), Vol. I, p. 523. Johns Hopkins, Baltimore, 1951; *Arch. Biochem.* **27,** 255 (1950).
12. T. L. Hill and M. F. Morales, *Arch. Biochem.* **29,** 450 (1950).
13. T. L. Hill and M. F. Morales, *J. Am. Chem. Soc.* **73,** 1656 (1951).
14. E. Baldwin, "Dynamic Aspects of Biochemistry," 3rd ed. Cambridge University Press, London and New York, 1957.
15. G. E. K. Branch and M. Calvin, "The Theory of Organic Chemistry," p. 44. Prentice-Hall, New York, 1945.
15a. R. J. Gillespie, G. A. Maw, and C. A. Vernon, *Nature* **171,** 1147 (1953).
16. G. N. Lewis and M. Randall, "Thermodynamics." McGraw-Hill, New York, 1923.
17. M. Johnson, *in* "Respiratory Enzymes" (H. A. Lardy, ed.), p. 255. Burgess, Minneapolis, 1949.
18. F. H. Johnson, H. Eyring, and M. J. Polissar, "The Kinetic Basis of Molecular Biology." Wiley, New York, 1954.
19. F. M. Huennekens, *in* "Technique in Organic Chemistry," Vol. VIII: Investigations of Rate and Mechanism of Reactions (A. Weissberger, ed.), p. 535. Interscience, New York, 1953.
20. I. M. Klotz, "Energetics in Biochemical Reactions." Academic Press, New York, 1957.
21. H. G. Bray and K. White, "Kinetics and Thermodynamics in Biochemistry." Churchill, London, 1957.
22. C. D. Coryell, *Science* **92,** 380 (1940).
23. N. O. Kaplan, *in* "The Enzymes" (J. B. Sumner and K. Myrbäck, eds.), Vol. II, Part 1, p. 55. Academic Press, New York, 1951.
24. K. Burton and H. A. Krebs, *Biochem. J.* **54,** 94 (1953).
25. K. Burton, *Biochem. J.* **59,** 44 (1955).
26. A. G. Ogston and O. Smithies, *Physiol. Revs.* **28,** 283 (1948).
27. H. A. Krebs and H. L. Kornberg, "Energy Transformations in Living Matter," p. 212. Springer, Berlin, 1957.
27a. L. B. Eggleston and R. Hems, *Biochem. J.* **52,** 156 (1952).
28. O. Meyerhof, *Ann. N. Y. Acad. Sci.* **45,** 377 (1945).
29. J. M. Sturtevant, *in* "Technique in Organic Chemistry," Vol. I: Physical Methods (A. Weissberger, ed.), p. 1. Interscience, New York, 1949.
30. A. Buzzell and J. M. Sturtevant, *J. Am. Chem. Soc.* **73,** 2454 (1951).
31. O. Meyerhof and K. Lohmann, *Biochem. Z.* **273,** 73 (1934).
32. O. Meyerhof and W. Schulz, *Biochem. Z.* **281,** 292 (1935).
33. T. Bücher, *Biochim. et Biophys. Acta* **1,** 292 (1947).
34. H. D. Kay, *Biochem. J.* **22,** 855 (1928).
35. S. P. Colowick, quoted by Kaplan (23), p. 58.
36. R. J. Podolsky and J. M. Sturtevant, *J. Biol. Chem.* **217,** 603 (1955).
37. R. A. Alberty, R. M. Smith, and R. M. Bock, *J. Biol. Chem.* **193,** 425 (1951).

38. R. J. Podolsky and M. F. Morales, *J. Biol. Chem.* **218,** 945 (1956).
39. N. S. Ging and J. M. Sturtevant, *J. Am. Chem. Soc.* **76,** 2087 (1954).
40. O. Meyerhof, R. Shatas, and A. Kaplan, *Biochim. et Biophys. Acta* **12,** 121 (1953).
41. L. Levintow and A. Meister, *J. Biol. Chem.* **209,** 265 (1954).
41a. H. Borsook, *Congr. intern. biochim., 2e Congr., Paris, 1952* p. 37 (1952).
42. M. F. Morales, J. Botts, J. J. Blum, and T. L. Hill, *Physiol. Revs.* **35,** 475 (1955).
43. K. Burton, *Biochem. J.* **71,** 388 (1959).
43a. T. H. Benzinger and R. Hems, *Proc. Natl. Acad. Sci. U. S.* **42,** 896 (1956).
43b. C. Kitzinger and R. Hems, *Biochem. J.* **71,** 395 (1959).
43c. T. H. Benzinger, C. Kitzinger, R. Hems, and K. Burton, *Biochem. J.* **71,** 400 (1959).
44. E. A. Robbins and P. D. Boyer, *J. Biol. Chem.* **224,** 121 (1957).
44a. O. Meyerhof and H. Green, *J. Biol. Chem.* **178,** 655 (1949).
44b. H. Theorell, *in* "Currents in Biochemical Research" (D. E. Green, ed.), p. 275. Interscience, New York, 1956.
44c. P. W. Robbins and F. Lipmann, *J. Biol. Chem.* **233,** 686 (1958).
45. O. Meyerhof and P. Oesper, *J. Biol. Chem.* **179,** 1371 (1949).
45a. I. Krimsky, *J. Biol. Chem.* **234,** 232 (1959).
45b. J. T. McQuate and M. F. Utter, *J. Biol. Chem.* **234,** 2151 (1959).
46. L. Noda, S. A. Kuby, and H. A. Lardy, *J. Biol. Chem.* **210,** 83 (1954).
47. W. P. Jencks, *Biochim. et Biophys. Acta* **24,** 227 (1957).
48. E. R. Stadtman, *in* "Mechanisms of Enzyme Action" (W. D. McElroy and B. Glass, eds.), p. 581. Johns Hopkins, Baltimore, 1954.
49. R. A. Smith, I. F. Frank, and I. C. Gunsalus, *Federation Proc.* **16,** 251 (1957).
49a. I. C. Gunsalus and R. A. Smith, *Proc. Intern. Symposium on Enzyme Chem., Tokyo and Kyoto, 1957* p. 77 (1958).
50. A. Kornberg, *Advances in Enzymol.* **18,** 191 (1957).
51. P. Berg and W. K. Joklik, *J. Biol. Chem.* **210,** 657 (1954).
52. H. A. Krebs and R. Hems, *Biochim. et Biophys. Acta* **12,** 172 (1953).
53. D. R. Sanadi, D. M. Gibson, P. Ayengar, and M. Jacob, *J. Biol. Chem.* **218,** 505 (1956).
54. P. Ayengar, D. M. Gibson, C. H. Lee Peng, and D. R. Sanadi, *J. Biol. Chem.* **218,** 521 (1956).
54a. J. L. Strominger, L. A. Heppel, and E. S. Maxwell, *Biochim. et Biophys. Acta* **32,** 412 (1959).
54b. L. A. Heppel, J. L. Strominger, and E. S. Maxwell, *Biochim. et Biophys. Acta* **32,** 422 (1959).
55. K. Lohmann, *Naturwissenschaften* **17,** 624 (1929).
56. C. H. Fiske and Y. SubbaRow, *Science* **70,** 381 (1929).
57. K. Lohmann and P. Shuster, *Biochem* Z. **282,** 104 (1953).
58. S. E. Kerr, *J. Biol. Chem.* **139,** 121 (1941).
59. G. A. LePage, *in* "Biochemical Preparations" (H. E. Carter, ed.), Vol I, p. 5. Wiley, New York, 1949.
60. L. Berger, *in* "Methods in Enzymology" (S. P. Colowick and N. O. Kaplan, eds.), Vol. III, p. 862. Academic Press, New York, 1957.
61. W. E. Cohn and C. E. Carter, *J. Am. Chem. Soc.* **72,** 4273 (1950).

62. R. B. Hurlbert, H. Schmitz, A. F. Brumm, and V. R. Potter, *J. Biol. Chem.* **209,** 23 (1954).
63. G. A. LePage, *in* "Biochemical Preparations" (H. E. Carter, ed.), Vol. I, p. 1. Wiley, New York, 1949.
64. L. Berger, *in* "Methods in Enzymology" (S. P. Colowick and N. O. Kaplan, eds.), Vol. III, p. 864. Academic Press, New York, 1957.
65. D. H. Marrian, *Biochim. et Biophys. Acta* **12,** 492 (1953); **13,** 278 (1954).
66. I. Lieberman, *J. Am. Chem. Soc.* **77,** 3373 (1955).
67. J. Baddiley and A. R. Todd, *J. Chem. Soc.* p. 648 (1947).
68. J. Baddiley, A. M. Michelson, and A. R. Todd, *J. Chem. Soc.* p. 582 (1949).
69. H. G. Khorana, *J. Am. Chem. Soc.* **76,** 3517 (1954).
69a. M. Smith and H. G. Khorana, *J. Am. Chem. Soc.* **80,** 1141 (1958).
70. R. H. Hall and H. G. Khorana, *J. Am. Chem. Soc.* **76,** 5056 (1954).
71. W. E. Cohn, *in* "Methods in Enzymology" (S. P. Colowick and N. O. Kaplan, eds.), Vol. III, p. 867. Academic Press, New York, 1957.
72. R. M. Bock, S. A. Morell, N. S. Ling, and S. H. Lipton, *Arch. Biochem. Biophys.* **62,** 253 (1956).
73. H. G. Albaum and R. Lipshitz, *Arch. Biochem.* **27,** 102 (1950).
74. H. G. Albaum, T. Cayle, and A. Shapiro, *J. Clin. Invest.* **30,** 525 (1951).
75. H. M. Kalckar, *J. Biol. Chem.* **167,** 445 (1947).
76. A. Munch-Petersen and H. M. Kalckar, *in* "Methods in Enzymology" (S. P. Colowick and N. O. Kaplan, eds.), Vol. III, p. 869. Academic Press, New York, 1957.
77. B. L. Strehler and W. D. McElroy, *in* "Methods in Enzymology" (S. P. Colowick and N. O. Kaplan, eds.), Vol. III, p. 871. Academic Press, New York, 1957.
78. N. O. Kaplan, *in* "Methods in Enzymology" (S. P. Colowick and N. O. Kaplan, eds.), Vol. III, p. 873. Academic Press, New York, 1957.
79. F. E. Hunter, *in* "Phosphorus Metabolism" (W. D. McElroy and B. Glass, eds.), Vol. I, p. 297. Johns Hopkins, Baltimore, Maryland, 1951.
80. D. E. Green, *Biol. Revs. Cambridge Phil. Soc.* **26,** 430 (1951).
81. A. L. Lehninger, *Harvey Lectures* **49,** 176 (1955).
82. D. E. Green and H. Beinert, *Ann. Rev. Biochem.* **24,** 16 (1955).
83. B. Chance and G. R. Williams, *Advances in Enzymol.* **17,** 65 (1956).
83a. A. L. Lehninger, C. L. Wadkins, C. Cooper, T. M. Devlin, and J. L. Gamble, Jr., *Science* **128,** 450 (1958).
84. C. Cooper and A. L. Lehninger, *J. Biol. Chem.* **219,** 489, 519 (1956).
85. T. M. Devlin and A. L. Lehninger, *J. Biol. Chem.* **219,** 507 (1956); **233,** 1586 (1958).
86. D. M. Ziegler, R. Lester, and D. E. Green, *Biochim. et Biophys. Acta* **21,** 80 (1956).
87. D. E. Green, R. Lester, and D. M. Ziegler, *Biochim. et Biophys. Acta* **23,** 516 (1957).
87a. D. E. Green and F. L. Crane, *Proc. Intern. Symposium on Enzyme Chem., Tokyo and Kyoto, 1957* p. 275 (1958).
88. W. C. McMurray, G. F. Maley, and H. A. Lardy, *J. Biol. Chem.* **230,** 219 (1958).
89. W. W. Kielley and J. R. Bronk, *J. Biol. Chem.* **230,** 521 (1958).
89a. W. W. Kielley and J. R. Bronk, *Proc. Intern. Symposium on Enzyme Chem., Tokyo and Kyoto, 1957* p. 286 (1958).

90. W. W. Kielley and R. K. Kielley, *J. Biol. Chem.* **191,** 485 (1951).
91. E. C. Slater, *Nature* **179,** 363 (1957).
91a. E. C. Slater, *Proc. Intern. Symposium on Enzyme Chem., Tokyo and Kyoto, 1957* p. 288 (1958).
91b. G. W. Drysdale and M. Cohn, *J. Biol. Chem.* **233,** 1574 (1959).
91c. P. D. Boyer, *Proc. Intern. Symposium on Enzyme Chem., Tokyo and Kyoto, 1957* p. 301 (1958).
91d. D. I. Arnon, F. R. Whatley, and M. B. Allen, *Science* **127,** 1026 (1958).
91e. D. I. Arnon, F. R. Whatley, and M. B. Allen, *Biochim. et Biophys. Acta* **32,** 47 (1959).
91f. A. W. Frenkel, *J. Biol. Chem.* **222,** 823 (1956).
91g. I. C. Anderson and R. C. Fuller, *Arch. Biochem. Biophys.* **76,** 168 (1958).
91h. W. Vishniac, B. L. Horecker, and S. Ochoa, *Adv. Enzymol.* **19,** 1 (1957).
91i. J. A. Bassham and M. Calvin, "The Path of Carbon in Photosynthesis." Prentice-Hall, Englewood Cliffs, New Jersey, 1957.
91j. H. Gaffron, A. H. Brown, C. S. French, R. Livingston, E. I. Rabinowitch, B. L. Strehler, and N. E. Tolbert, eds., "Research in Photosynthesis." Interscience, New York, 1957.
91k. F. R. Whatley, M. B. Allen, L. L. Rosenberg, J. B. Capindale, and D. I. Arnon, *Biochim. et Biophys. Acta* **20,** 462 (1956).
91l. F. R. Whatley, M. B. Allen, and D. I. Arnon, *Biochim. et Biophys. Acta* **32,** 32 (1959).
91m. M. Avron and A. T. Jagendorf, *J. Biol. Chem.* **234,** 967 (1959).
91n. L. P. Vernon, *J. Biol. Chem.* **233,** 212 (1958).
92. V. Henning, E. M. Möslein, and F. Lynen, *Arch. Biochem. Biophys.* **83,** 259 (1959).
92a. K. Bloch, S. Chaykin, A. H. Phillips, and A. de Waard, *J. Biol. Chem.* **234,** 2595 (1959).
92b. A. Kornberg, I. Lieberman, and E. S. Simms, *J. Biol. Chem.* **215,** 389 (1955).
93. H. Schmitz, R. B. Hurlbert, and V. R. Potter, *J. Biol. Chem.* **209,** 41 (1954).
94. H. Schmitz, W. Hart, and H. Ried, *Z. Krebsforsch.* **60,** 301 (1955).
95. H. Schmitz, V. R. Potter, and R. B. Hurlbert, *Z. Krebsforsch.* **60,** 419 (1955).
95a. H. Schmitz, V. R. Potter, R. B. Hurlbert, and D. M. White, *Cancer Research* **14,** 66 (1954).
95b. K. K. Tsuboi, *Arch. Biochem. Biophys.* **83,** 445 (1959).
96. E. E. B. Smith and G. T. Mills, *Biochim. et Biophys. Acta* **13,** 587 (1954).
97. B. J. Vanderheiden, F. M. Huennekens, and B. W. Gabrio, *Federation Proc.* **17,** 327 (1958).
97a. C. Bishop, D. M. Rankine, and J. H. Talbott, *J. Biol. Chem.* **234,** 1233 (1959).
97b. G. C. Mills and L. B. Summers, *Arch. Biochem. Biophys.* **84,** 7 (1959).
97c. G. R. Bartlett, *J. Biol. Chem.* **234,** 449 (1959).
98. E. Cabib and L. F. Leloir, *J. Biol. Chem.* **206,** 779 (1954).
99. S. H. Lipton, S. A. Morell, and A. Frieden, *J. Am. Chem. Soc.* **75,** 5449 (1953).
100. H. Schmitz, *Biochem. Z.* **325,** 555 (1954).
101. J. Baddiley and A. P. Mathias, *J. Chem. Soc.* p. 2723 (1954).
102. T. Hultin, *Exptl. Cell Research* **12,** 413–415 (1957).

103. F. P. W. Winteringham, P. M. Bridges, and G. C. Hellyer, *Biochem. J.* **59,** 13 (1955).
104. B. Sacktor and D. G. Cochran, *J. Biol. Chem.* **226,** 241 (1957).
105. C. Rey, *Biochim. et Biophys. Acta* **19,** 300 (1956).
106. P. C. Caldwell, *Biochem. J.* **55,** 458 (1953).
107. T. Saito, K. I. Arai, and T. Tanaka, *Nature* **181,** 1127 (1958).
108. T. Saito and K. I. Arai, *Nature* **179,** 820 (1957).
109. G. Schmidt, *in* "Phosphorus Metabolism" (W. D. McElroy and B. Glass, eds.), Vol. I, p. 443. Johns Hopkins, Baltimore, Maryland, 1951.
110. A. Kornberg, S. R. Kornberg, and E. S. Simms, *Biochim. et Biophys. Acta* **20,** 215 (1956).
111. L. Liebermann, *Ber. deut. chem. Ges.* **21,** 598 (1888).
112. G. Schmidt and S. J. Thannhauser, *J. Biol. Chem.* **161,** 83 (1945).
113. J. M. Wiame, *Biochim. et Biophys. Acta* **1,** 234 (1947).
114. G. Schmidt, L. Hecht, and S. J. Thannhauser, *J. Biol. Chem.* **166,** 775 (1946).
115. J. M. Wiame, *J. Biol. Chem.* **178,** 919 (1949).
115a. S. P. Damle and P. S. Krishnan, *Arch. Biochem. Biophys.* **49,** 58 (1954).
115b. K. K. Tewari and P. S. Krishnan, *Arch. Biochem. Biophys.* **82,** 99 (1959).
116. E. Juni, M. D. Kamen, S. Spiegelman, and J. M. Wiame, *Nature* **160,** 717 (1947).
117. E. Juni, M. D. Kamen, J. M. Reiner, and S. Spiegelman, *Arch. Biochem.* **18,** 387 (1948).
118. B. J. Katchman and W. O. Fetty, *J. Bacteriol.* **69,** 607 (1955).
119. T. V. Peters and W. Rieman, *Anal. Chim. Acta* **14,** 131 (1956).
120. H. Deuel, J. Salms, and L. Anyas-Weisz, *Helv. Chim. Acta* **33,** 2171 (1950).
121. J. P. Crowther, *Nature* **173,** 486 (1954).
122. H. Mattenheimer, *Z. physiol. Chem.* **303,** 125 (1956).
123. I. Mandl and C. Neuberg, *in* "Methods in Enzymology" (S. F. Colowick and N. O. Kaplan, eds.), Vol. II, p. 577. Academic Press, New York, 1955.
124. A. Kornberg, *J. Biol. Chem.* **182,** 779 (1950).
125. D. R. Briggs, *J. Biol. Chem.* **132,** 261 (1940).
126. J. R. VanWazer and D. Campanella, *J. Am. Chem. Soc.* **72,** 655 (1950).
127. B. Ingelman and H. Malmgren, *Acta. Chem. Scand.* **3,** 157 (1947).
128. L. Michaelis, *Cold Spring Harbor Symp. Quant. Biol.* **12,** 143 (1947).
128a. L. Lison, *Arch. biol. (Liège)* **46,** 599 (1935).
128b. O. Necas, *Exptl. Cell Research* **14,** 216 (1958).
128c. T. Caspersson and K. Brandt, *Protoplasma* **35,** 507 (1941).
128d. S. Mudd, A. Yoshida, and M. Koike, *J. Bacteriol.* **75,** 224 (1958).
128e. D. D. Hickman and A. W. Frenkel, *J. Biophys. Biochem. Cytol.* **6,** 277 (1959).
129. F. T. Kenney, S. P. Colowick, and E. Barbehenn, *Arch. Biochem. Biophys.* **69,** 617 (1957).
130. H. Mattenheimer, *Z. physiol. Chem.* **303,** 107, 115 (1956).
131. I. Lieberman, *J. Biol. Chem.* **219,** 307 (1956).
131a. S. R. Kornberg, I. R. Lehman, M. J. Bessman, E. S. Simms, and A. Kornberg, *J. Biol. Chem.* **233,** 159 (1958).
131b. G. W. Rafter, *Arch. Biochem. Biophys.* **81,** 238 (1959).
132. R. Jeener and J. Brachet, *Enzymologia* **11,** 222 (1944).

133. G. Schmidt, L. Hecht, and S. J. Thannhauser, *J. Biol. Chem.* **178,** 733 (1949).
134. G. Schmidt, K. Serdarian, L. M. Greenbaum, M. D. Hickey, and S. J. Thannhauser, *Biochim. et Biophys. Acta* **20,** 135 (1956).
134a. T. Sall, S. Mudd, and J. C. Davis, *Arch. Biochem. Biophys.* **60,** 130 (1956).
135. T. Sall, S. Mudd, and A. Takagi, *J. Bacteriol.* **76,** 640 (1958).
135a. B. J. Katchman, W. O. Fetty, and K. A. Busch, *J. Bacteriol.* **77,** 331 (1959).
135b. R. Chayen, S. Chayen, and E. R. Roberts, *Biochim. et Biophys. Acta* **16,** 117 (1955).
135c. P. S. Krishnan, S. P. Damle, and V. Bajaj, *Arch. Biochem. Biophys.* **67,** 35 (1957).
136. C. C. Lindegren, *Nature* **169,** 63 (1947).
137. A. Yoshida and A. Yamataka, *J. Biochem. (Tokyo)* **40,** 85 (1953).
138. A. Yoshida, *J. Biochem. (Tokyo)* **42,** 163 (1955).
139. O. Hofmann-Ostenhof, J. Kenedy, K. Keck, O. Gabriel, and H. W. Schönfellinger, *Biochim. et Biophys. Acta* **14,** 285 (1954).
139a. O. Hoffman-Ostenhof and L. Slechta, *Proc. Intern. Symposium on Enzyme Chem., Tokyo and Kyoto, 1957* p. 180 (1958).
140. S. R. Kornberg, *Biochim. et Biophys. Acta* **26,** 294 (1957).
141. J. P. Ebel, *Compt. rend.* **228,** 1312 (1949).
142. J. F. G. M. Wintermans, *Proc. Koninkl. Ned. Akad. Wetenschap.* **C57,** 574 (1954).
143. W. F. Nickerson, *Experientia* **5,** 202 (1949).
144. J. P. Ebel and L. Mehr, *Bull. soc. chim. biol.* **39,** 1535 (1957).
145. B. Ingelman and H. Malmgren, *Acta Chem. Scand.* **1,** 422 (1947).
146. B. Ingelman and H. Malmgren, *Acta Chem. Scand.* **2,** 365 (1949).
146a. W. S. Pierpoint, *Biochem. J.* **65,** 67 (1957).
146b. A. Muhammed, A. Rodgers, and D. E. Hughes, *J. Gen. Microbiol.* **20,** 482 (1959).
146c. S. R. Kornberg, *J. Biol. Chem.* **218,** 23 (1956).
147. L. A. Heppel, *in* "Methods in Enzymology" (S. P. Colowick and N. O. Kaplan, eds.), Vol. II, p. 570. Academic Press, New York, 1955.
148. F. Winder and J. M. Denneny, *Nature* **175,** 636 (1955).
149. H. Holzer, *Z. Naturforsch.* **6b,** 424 (1951).
150. H. Stich, *Z. Naturforsch.* **8b,** 36 (1953).
150a. I. S. Kulaev and A. N. Belozersky, *Biokhimiya* **22,** 29 (1957).
150b. A. N. Belozersky and I. S. Kulaev, *Biokhimiya* **22,** 587 (1957).
151. S. L. Friess, *J. Am. Chem. Soc.* **75,** 323 (1953).
152. E. T. Friess and M. F. Morales, *Arch. Biochem. Biophys.* **56,** 326 (1955).
153. C. C. Lindegren, "The Yeast Cell," p. 29. Educational Publishers, St. Louis, Missouri, 1949.
154. W. Vishniac, *Arch. Biochem.* **26,** 167 (1950).
155. S. Niermerko, *Acta Biol. Exptl. (Lodz)* **15,** 91 (1950).
156. S. Niemerko and W. Niemerko, *Acta Biol. Exptl. (Lodz)* **15,** 111 (1950).
157. S. Niemerko and W. Niemerko, *Nature* **166,** 268 (1950).
158. A. B. Wojtczak, *Acta Biol. Exptl. (Lodz)* **17,** 235 (1956).
159. W. S. Pierpoint, *Biochem. J.* **67,** 624 (1957).
160. J. Heller, St. Karpiak, and I. Zabikova, *Nature* **166,** 187 (1950).
160a. J. Heller, T. Chojnacki, and M. Piechowska, *Bull. acad. polon. sci.* **6,** 7 (1958).

160b. J. Heller and M. Piechowska, *Bull. acad. polon. sci.* **6,** 187 (1958).
161. H. J. Koepsell, M. J. Johnson, and J. S. Meek, *J. Biol. Chem.* **154,** 535 (1944).
162. E. R. Stadtman and H. A. Barker, *J. Biol. Chem.* **184,** 769 (1950).
163. H. R. Whiteley, *Proc. Natl. Acad. Sci. U.S.* **39,** 772, 779 (1953).
164. J. M. Lowenstein and P. P. Cohen, *J. Am. Chem. Soc.* **76,** 5571 (1954).
164a. R. L. Metzenberg, M. Marshall, and P. P. Cohen, *J. Biol. Chem.* **233,** 1560 (1958).
164b. M. E. Jones, L. Spector, and F. Lipmann, *Congr. intern. biochim., 3e Congr., Brussels, Belgium, 1955* p. 67 (1956).
165. M. E. Jones, L. Spector, and F. Lipmann, *J. Am. Chem. Soc.* **77,** 819 (1955).
165a. P. Reichard, *Adv. in Enzymol.* **21,** 263 (1959).
166. S. Black and N. G. Wright, *J. Biol. Chem.* **213,** 27, 38 (1955).
167. M. Flavin, H. Castro-Mendoza, and S. Ochoa, *Biochim. et Biophys. Acta* **20,** 591 (1956).
168. M. Cohn, *in* "Phosphorus Metabolism" (W. D. McElroy and B. Glass, eds.), Vol. I, p. 374. Johns Hopkins, Baltimore, Maryland, 1951.
169. S. Kaufman, *J. Biol. Chem.* **216,** 153 (1955).
170. W. H. Elliott, *J. Biol. Chem.* **201,** 661 (1953).
171. F. Lipmann, *Federation Proc.* **1,** 122 (1942).
172. F. Lipmann, *J. Biol. Chem.* **155,** 55 (1944).
173. F. Lipmann, *Cold Spring Harbor Symposia Quant. Biol.* **7,** 248 (1939).
174. H. J. Koepsell and M. J. Johnson, *J. Biol. Chem.* **145,** 379 (1942).
175. G. Kalnitsky and C. H. Werkman, *Arch. Biochem.* **2,** 113 (1943).
176. M. F. Utter and C. H. Werkman, *Arch. Biochem.* **5,** 413 (1944).
177. F. Lipmann, *Advances in Enzymol.* **6,** 231 (1946).
178. F. Lynen, *Ber. deut. chem. Ges.* **73,** 367 (1940).
179. F. Lipmann and L. C. Tuttle, *J. Biol. Chem.* **153,** 571 (1944).
180. E. R. Stadtman and F. Lipmann, *J. Biol. Chem.* **185,** 549 (1950).
181. E. R. Stadtman, *in* "Methods in Enzymology" (S. P. Colowick and N. O. Kaplan, eds.), Vol. III, p. 228. Academic Press, New York, 1957.
182. A. W. D. Avison, *J. Chem. Soc.* p. 732 (1955).
183. F. Lipmann and L. C. Tuttle, *J. Biol. Chem.* **159,** 21 (1945).
184. N. O. Kaplan and F. Lipmann, *Federation Proc.* **7,** 163 (1948).
185. I. A. Rose *in* "Methods in Enzymology" (S. P. Colowick and N. O. Kaplan, eds.), Vol. I, p. 591. Academic Press, New York, 1955.
186. I. A. Rose, M. Grunberg-Manago, S. R. Korey, and S. Ochoa, *J. Biol. Chem.* **211,** 737 (1954).
187. E. R. Stadtman, G. D. Novelli, and F. Lipmann, *J. Biol. Chem.* **191,** 365 (1951).
188. E. R. Stadtman, *J. Biol. Chem.* **196,** 527, 535 (1952).
189. E. R. Stadtman, *in* "Methods in Enzymology" (S. P. Colowick and N. O. Kaplan, eds.), Vol. I, p. 596. Academic Press, New York, 1955.
190. D. S. Goldman, *Biochim. et Biophys. Acta* **28,** 436 (1958).
191. J. R. Stern and S. Ochoa, *J. Biol. Chem.* **191,** 161 (1951).
191a. T. Ramakrishnan, *Arch. Biochem. Biophys.* **81,** 525 (1959).
192. I. C. Gunsalus, B. L. Horecker, and W. A. Wood, *Bacteriol. Revs.* **19,** 80 (1955).
193. L. P. Hager, D. M. Geller, and F. Lipmann, *Federation Proc.* **13,** 734 (1954).

194. J. Harting, *Federation Proc.* **10,** 195 (1951).
195. I. Krimsky and E. Racker, *J. Biol. Chem.* **198,** 721, 731 (1952).
196. J. Harting and S. Velick, *Federation Proc.* **11,** 226 (1952).
197. E. Racker, *in* "Mechanism of Enzyme Action" (W. D. McElroy and B. Glass, eds.), p. 464. Johns Hopkins Press, Baltimore, Maryland, 1954.
198. E. C. Heath, J. Hurwitz and B. L. Horecker, *J. Am. Chem. Soc.* **78,** 5449 (1956); E. C. Heath, J. Hurwitz, B. L. Horecker, and A. Ginsburg, *J. Biol. Chem.* **231,** 1009 (1958).
199. H. Green and O. Meyerhof, *J. Biol. Chem.* **197,** 347 (1952).
200. B. Axelrod, *Advances in Enzymol.* **17,** 159 (1956).
201. B. Shapiro and E. Wertheimer, *Nature* **156,** 690 (1945).
202. A. Lehninger, *J. Biol. Chem.* **162,** 333 (1946).
202a. S. Grisolia, J. Caravaca, and B. K. Joyce, *Biochim. et Biophys. Acta* **29,** 432 (1958).
203. E. Negelein and H. Brömel, *Biochem. Z.* **301,** 135 (1939); **303,** 132 (1939).
204. O. Warburg and W. Christian, *Biochem. Z.* **303,** 40 (1939).
205. E. Negelein, *in* "Methods in Enzymology" (S. P. Colowick and N. O. Kaplan, eds.), Vol. III, p. 216. Academic Press, New York, 1957.
206. O. Warburg and W. Christian, *Biochem. Z.* **301,** 221 (1939).
207. E. G. Krebs, G. W. Rafter, and J. M. Junge, *J. Biol. Chem.* **200,** 479 (1953).
208. G. T. Cori, M. W. Slein, and C. F. Cori, *J. Biol. Chem.* **173,** 605 (1948).
209. S. Velick, *in* "Methods in Enzymology" (S. P. Colowick and N. O. Kaplan, eds.), Vol. I, p. 401. Academic Press, New York, 1955.
210. M. Gibbs, *Nature* **170,** 164 (1952).
211. D. I. Arnon, *Science* **116,** 635 (1952).
212. P. K. Stumpf, *J. Biol. Chem.* **182,** 261 (1950).
213. P. Berg, *J. Am. Chem. Soc.* **77,** 3163 (1955).
214. P. Berg, *J. Biol. Chem.* **222,** 991, 1015 (1956).
215. P. T. Talbert and F. M. Huennekens, *J. Am. Chem. Soc.* **78,** 4671 (1956).
216. C. H. Lee Peng, *Biochim. et Biophys. Acta* **22,** 42 (1956).
217. W. P. Jencks and F. Lipmann, *J. Biol. Chem.* **225,** 207 (1957).
218. M. Whitehouse, H. Moeski, and S. Gurin, *J. Biol. Chem.* **226,** 813 (1957).
219. H. S. Moyed and F. Lipmann, *J. Bacteriol.* **73,** 177 (1957).
220. M. B. Hoagland, *Biochim. et Biophys. Acta* **16,** 288 (1955).
221. J. A. DeMoss and G. D. Novelli, *Biochim. et Biophys. Acta* **18,** 592 (1955).
222. P. Berg, *J. Biol. Chem.* **222,** 1025 (1956).
223. D. Schachter and J. V. Taggart, *J. Biol. Chem.* **203,** 925 (1953); **208,** 263 (1954).
224. K. Moldave and A. Meister, *J. Biol. Chem.* **229,** 463 (1957).
225. G. M. Kellerman, *J. Biol. Chem.* **231,** 427 (1958).
226. P. W. Robbins and F. Lipmann, *J. Am. Chem. Soc.* **78,** 2652, 6409 (1956).
227. P. W. Robbins and F. Lipmann, *J. Biol. Chem.* **229,** 837 (1957).
228. R. S. Bandurski, L. G. Wilson, and C. L. Squires, *J. Am. Chem. Soc.* **78,** 6408 (1956).
229. L. G. Wilson and R. S. Bandurski, *Arch. Biochem. Biophys.* **62,** 503 (1956).
229a. L. G. Wilson and R. S. Bandurski, *J. Biol. Chem.* **233,** 975 (1958).
229b. R. S. Bandurski and L. G. Wilson, *Proc. Intern. Symposium on Enzyme Chem., Tokyo and Kyoto, 1957* p. 92 (1958).
230. H. Hilz and F. Lipmann, *Proc. Natl. Acad. Sci. U.S.* **41,** 880 (1955).
231. F. D'Abramo and F. Lipmann, *Biochim. et Biophys. Acta* **25,** 211 (1957).
232. F. Lipmann, *Science* **128,** 575 (1958).

232*a*. P. W. Robbins and F. Lipmann, *J. Biol. Chem.* **233,** 681 (1958).
233. L. J. Reed, F. R. Leach, M. Koike, and M. E. Levitch, *Federation Proc.* **16,** 236 (1957).
234. W. K. Maas, *Résumés communs. 3rd Intern. Congr. Biochem., Brussels* p. 32 (1955).
235. R. L. Airth, W. C. Rhodes, and W. D. McElroy, *Biochim. et Biophys. Acta* **27,** 519 (1958).
235*a*. W. C. Rhodes and W. D. McElroy, *J. Biol. Chem.* **233,** 1528 (1958).
235*b*. N. Katunuma, *Arch. Biochem. Biophys.* **76,** 547 (1958).
235*c*. N. Ellfolk and N. Katunuma, *Arch. Biochem. Biophys.* **81,** 521 (1959).
235*d*. P. V. Vignais and I. Zabin, *Biochim. et Biophys. Acta* **29,** 263 (1958).
236. P. Castelfranco, K. Moldave, and A. Meister, *J. Am. Chem. Soc.* **80,** 2335 (1958).
236*a*. P. Berg, *J. Biol. Chem.* **233,** 608 (1958).
236*b*. D. J. McCorquodale and G. C. Mueller, *Arch. Biochem. Biophys.* **77,** 13 (1958).
236*c*. K. Moldave, P. Castelfranco, and A. Meister, *J. Biol. Chem.* **234,** 841 (1959).
237. J. A. DeMoss, S. M. Genuth, and G. D. Novelli, *Proc. Natl. Acad. Sci. U.S.* **42,** 325 (1956).
237*a*. P. Berg, *J. Biol. Chem.* **233,** 601 (1958).
238. M. Karasek, P. Castelfranco, P. R. Krishnaswamy, and A. Meister, *J. Am. Chem. Soc.* **80,** 2335 (1958).
238*a*. H. S. Kingdon, L. T. Webster, Jr., and E. W. Davie, *Proc. Natl. Acad. Sci. U. S.* **44,** 757 (1958).
238*b*. S. B. Weiss, H. G. Zachau, and F. Lipmann, *Arch. Biochem. Biophys.* **83,** 101 (1959).
238*c*. M. Karasek, P. R. Krishnaswamy, P. Castelfranco, and A. Meister, *in* "Microsomal Proteins and Protein Synthesis" (R. B. Roberts, ed.), p. 109. Pergamon Press, New York, 1958.
239. P. C. Zamecnik, M. L. Stephenson, and L. I. Hecht, *Proc. Natl. Acad. Sci. U.S.* **44,** 73 (1958).
239*a*. L. T. Webster, Jr. and E. W. Davie, *Biochim. et Biophys. Acta* **35,** 559 (1959).
240. M. B. Hoagland, P. C. Zamecnik, N. Sharon, F. Lipmann, M. P. Stulberg, and P. D. Boyer, *Biochim. et Biophys. Acta* **26,** 215 (1957).
241. P. D. Boyer and M. P. Stulberg, *Proc. Natl. Acad. Sci. U.S.* **44,** 92 (1958).
242. P. Berg and E. J. Ofengand, *Proc. Natl. Acad. Sci. U.S.* **44,** 78 (1958).
243. M. B. Hoagland, E. B. Keller, and P. C. Zamecnik, *J. Biol. Chem.* **218,** 345 (1956).
244. B. K. Bachhawat, W. G. Robinson, and M. J. Coon, *J. Biol. Chem.* **219,** 539 (1956).
244*a*. M. J. Coon and F. P. Kupiecki, *J. Biol. Chem.* **234,** 2428 (1959).
244*b*. F. Lynen, J. Knappe, E. Lorch, G. Jutting, and E. Ringelmann, *Angew. Chem.* **71,** 481 (1959).
244*c*. F. Lynen, *J. Cellular & Comp. Physiol.* (in press).
244*d*. S. J. Wakil, *J. Am. Chem. Soc.* **80,** 6465 (1958).
244*e*. S. J. Wakil, E. B. Titchener, and D. M. Gibson, *Biochim. et Biophys. Acta* **29,** 225 (1958).
245. G. D. Kalyankar and A. Meister, *J. Am. Chem. Soc.* **81,** 1515 (1959).
245*a*. J. Baddiley, J. G. Buchanan, and R. Letters, *J. Chem. Soc.* p. 1067 (1957).

245b. P. Reichard and N. R. Ringertz, *J. Am. Chem. Soc.* **81,** 878 (1959).
245c. L. G. Wilson and R. S. Bandurski, *J. Am. Chem. Soc.* **80,** 5576 (1958).
245d. H. D. Peck, Jr., *Proc. Natl. Acad. Sci. U.S.* **45,** 701 (1959).
245e. J. B. Ragland, *Arch. Biochem. Biophys.* **84,** 541 (1959).
246. G. D. Novelli and J. A. DeMoss, *J. Cellular & Comp. Physiol.* **50,** Suppl. 1, 173 (1957).
247. G. D. Novelli, *Proc. Natl. Acad. Sci. U.S.* **44,** 86 (1958).
248. J. A. DeMoss and G. D. Novelli, *Biochim. et Biophys. Acta* **22,** 49 (1956).
248a. B. Nisman, *Biochim. et Biophys. Acta* **32,** 18 (1959).
248b. J. Baddiley and F. C. Neuhaus, *Biochim. et Biophys. Acta* **33,** 277 (1959).
249. R. S. Schweet, F. C. Bovard, E. Allen, and E. Glassman, *Proc. Natl. Acad. Sci. U.S.* **44,** 173 (1958).
250. S. B. Weiss, G. Acs, and F. Lipmann, *Proc. Natl. Acad. Sci. U.S.* **44,** 189 (1958).
251. F. Lipmann, *Proc. Natl. Acad. Sci. U.S.* **44,** 67 (1958).
252. R. S. Schweet, R. W. Holley, and E. H. Allen, *Arch. Biochem. Biophys.* **71,** 311 (1957).
252a. R. S. Schweet and E. H. Allen, *J. Biol. Chem.* **233,** 1104 (1958).
252b. A. M. van de Ven, V. V. Koningsberger, and J. T. G. Overbeek, *Biochim. et Biophys. Acta* **28,** 134 (1958).
253. E. Scarano and R. Maggio, *Exptl. Cell Research* **12,** 403 (1957).
254. B. Nisman, F. H. Bergmann, and P. Berg, *Biochim. et Biophys. Acta* **26,** 639 (1957).
255. G. C. Webster, *in* "Chemical Basis of Heredity" (W. D. McElroy and B. Glass, eds.), p. 268. Johns Hopkins, Baltimore, 1957.
256. J. Mager and F. Lipmann, *Proc. Natl. Acad. Sci. U.S.* **44,** 305 (1958).
257. G. C. Webster, *Biochim. et Biophys. Acta* **20,** 565 (1956).
258. E. F. Gale, *Biochem. Soc. Symposia* **14,** 47 (1957).
258a. G. C. Webster, *Arch. Biochem. Biophys.* **82,** 125 (1959).
259. E. W. Davie, V. V. Koningsberger, and F. Lipmann, *Arch. Biochem. Biophys.* **65,** 21 (1956).
260. R. W. Holley and J. Goldstein, *J. Biol. Chem.* **234,** 1765 (1959).
260a. K. Ogata and H. Nohara, *Biochim. et Biophys. Acta* **25,** 659 (1957).
261. M. B. Hoagland, P. C. Zamecnik, and M. L. Stephenson, *Biochim. et Biophys. Acta* **24,** 215 (1957).
261a. L. I. Hecht, M. L. Stephenson, and P. C. Zamecnik, *Biochim. et Biophys. Acta* **29,** 260 (1958).
261b. H. G. Zachau, G. Acs, and F. Lipmann, *Proc. Natl. Acad. Sci. U.S.* **44,** 885 (1958).
261c. L. I. Hecht, M. L. Stephenson, and P. C. Zamecnik, *Proc. Natl. Acad. Sci. U.S.* **45,** 505 (1959).
262. E. B. Keller and P. C. Zamecnik, *J. Biol. Chem.* **221,** 45 (1956).
262a. J. Preiss, P. Berg, E. J. Ofengand, F. H. Bergmann, and M. Dieckmann, *Proc. Natl. Acad. Sci. U.S.* **45,** 319 (1959).
263. K. Lohmann and O. Meyerhof, *Biochem. Z.* **273,** 60 (1934).
264. R. S. Bandurski and B. Axelrod, *J. Biol. Chem.* **193,** 405 (1951).
265. C. S. Hanes and F. A. Isherwood, *Nature* **164,** 1107 (1949).
266. A. A. Benson, J. A. Bassham, M. Calvin, T. C. Goodale, V. A. Haas, and W. Stepka, *J. Am. Chem. Soc.* **72,** 1710 (1950).
267. W. Kiessling, *Ber. deut. chem. Ges.* **68,** 597 (1935).

268. W. Kiessling, *Ber. deut. chem. Ges.* **69,** 2331 (1936).
269. G. Schmidt and S. J. Thannhauser, *J. Biol. Chem.* **149,** 369 (1943).
270. P. Ohlmeyer, *J. Biol. Chem.* **190,** 21 (1951).
271. E. Baer and H. O. L. Fischer, *J. Biol. Chem.* **180,** 145 (1949).
272. E. Baer, *Biochem. Preparations* **2,** 25 (1952).
273. M. L. Utter and K. Kurahashi, *J. Biol. Chem.* **207,** 821 (1954).
273a. E. Baer, L. J. Ciplijanskas, and T. Visser, *J. Biol. Chem.* **234,** 1 (1959).
274. G. Schmidt, *in* "Methods in Enzymology" (S. P. Colowick and N. O. Kaplan, eds.), Vol. III, p. 223. Academic Press, New York, 1957.
275. J. A. Muntz and J. Hurwitz, *Arch. Biochem. Biophys.* **32,** 137 (1951).
276. O. Warburg and W. Christian, *Biochem. Z.* **310,** 384 (1941).
277. T. Bücher, *in* "Methods in Enzymology" (S. P. Colowick and N. O. Kaplan, eds.), Vol. I, p. 427. Academic Press, New York, 1955.
278. C. H. Werkman and H. G. Wood, *Advances in Enzymol.* **2,** 135 (1942).
279. M. F. Utter and H. G. Wood, *Advances in Enzymol.* **12,** 41 (1951).
280. L. O. Krampitz and C. H. Werkman, *Biochem. J.* **35,** 595 (1941).
281. L. O. Krampitz, H. G. Wood, and C. H. Werkman, *J. Biol. Chem.* **147,** 243 (1943).
282. G. W. E. Plaut and H. A. Lardy, *J. Biol. Chem.* **180,** 13 (1949).
283. I. R. McManus, *J. Biol. Chem.* **188,** 729 (1951).
284. M. F. Utter and H. G. Wood, *J. Biol. Chem.* **164,** 455 (1946).
285. M. F. Utter, *in* "Phosphorus Metabolism" (W. D. McElroy and B. Glass, eds.), Vol. I, p. 646. Johns Hopkins, Baltimore, 1951.
286. M. F. Utter and K. Kurahashi, *J. Am. Chem. Soc.* **75,** 758 (1951).
287. M. F. Utter and K. Kurahashi, *J. Biol. Chem.* **207,** 787 (1954).
288. M. F. Utter, K. Kurahashi, and I. A. Rose, *J. Biol. Chem.* **207,** 803 (1954).
289. W. Bartley, *Biochem. J.* **61,** 555 (1955).
290. P. Berg and W. K. Joklik, *Nature* **172,** 1008 (1953).
291. R. S. Bandurski and F. Lipmann, *Federation Proc.* **14,** 176 (1955).
292. K. Kurahashi and M. F. Utter, *Federation Proc.* **14,** 240 (1955).
292a. J. Cannata and A. O. M. Stoppani, *Biochim. et Biophys. Acta* **32,** 284 (1959).
293. R. S. Bandurski and C. M. Greiner, *J. Biol. Chem.* **204,** 781 (1953).
294. W. Bartley, *Biochem. J.* **56,** 387 (1954).
295. T. T. Chen, F. A. Loewus, and B. Vennesland, *J. Biol. Chem.* **213,** 547 (1955).
296. F. Kubowitz and P. Ott, *Biochem. Z.* **317,** 193 (1944).
297. A. Kornberg and W. E. Pricer, Jr., *J. Biol. Chem.* **193,** 481 (1951).
298. J. F. Kachmar and P. D. Boyer, *J. Biol. Chem.* **200,** 669 (1953).
299. J. L. Strominger, *Biochim. et Biophys. Acta* **16,** 616 (1955).
300. S. Ochoa, A. H. Mehler, and A. Kornberg, *J. Biol. Chem.* **174,** 979 (1948).
301. T. T. Chen and B. Vennesland, *J. Biol. Chem.* **213,** 533 (1955).
302. P. H. Abelson, E. Bolton, R. Britten, D. B. Cowie, and R. B. Roberts, *Proc. Natl. Acad. Sci. U.S.* **39,** 1020 (1953).
303. M. Calvin, *Proc. 3rd Intern. Congr. Biochem., Brussels* p. 211 (1955).
304. M. Santer and W. Vishniac, *Biochim. et Biophys. Acta* **18,** 157 (1955).
305. P. A. Trudinger, *Biochem. J.* **64,** 274 (1956).
306. F. H. Bergmann, J. C. Towne, and R. H. Burris, *J. Biol. Chem.* **230,** 13 (1958).
306a. I. Suzuki and C. H. Werkman, *Arch. Biochem. Biophys.* **77,** 112 (1958).

307. A. O. M. Stoppani, R. C. Fuller, and M. Calvin, *J. Bacteriol.* **69,** 491 (1955).
308. I. Suzuki and C. H. Werkman, *Arch. Biochem. Biophys.* **72,** 514 (1957).
309. J.-P. Aubert, G. Milhaud, and J. Millet, *Compt. rend.* **244,** 398 (1957).
309a. I. Suzuki and C. H. Werkman, *Arch. Biochem. Biophys.* **76,** 103 (1958).
310. J. Judis, H. Koffler, and D. M. Powelson, *Proc. Soc. Exptl. Biol. Med.* **89,** 665 (1955).
311. R. S. Bandurski, *J. Biol. Chem.* **217,** 137 (1955).
312. D. A. Walker, *Biochem. J.* **67,** 73 (1957).
313. D. A. Walker and J. M. A. Brown, *Biochem. J.* **67,** 79 (1957).
314. R. S. Bandurski and F. Lipmann, *J. Biol. Chem.* **219,** 741 (1956).
315. H. M. Kalckar, *Biochem. J.* **33,** 631 (1939).
316. L. F. Leloir and J. M. Munoz, *J. Biol. Chem.* **153,** 53 (1944).
317. G. H. Mudge, H. W. Neuberg, and S. W. Stanbury, *J. Biol. Chem.* **210,** 965 (1954).
318. E. B. Kalan and P. R. Srinivasan, *in* "Symposium on Amino Acid Metabolism" (W. D. McElroy and B. Glass, eds.), p. 826. Johns Hopkins, Baltimore, 1955.
319. P. R. Srinivasan, M. Katagiri, and D. B. Sprinson, *J. Am. Chem. Soc.* **77,** 4943 (1955).
320. P. R. Srinivasan and D. B. Sprinson, *Federation Proc.* **15,** 360 (1956).
320a. D. H. Levin and E. Racker, *J. Biol. Chem.* **234,** 2532 (1959).
320b. E. W. Fager, *Biochem. J.* **57,** 264 (1954).
320c. V. M. Clark, G. W. Kirby, and A. R. Todd, *Nature* **181,** 1650 (1958).
320d. A. R. Todd, *Proc. Natl. Acad. Sci. U.S.* **45,** 1389 (1959).
320e. A. F. Brodie and B. R. Davis, *Federation Proc.* **18,** 778 (1959).
321. A. O. M. Stoppani, S. L. S. de Favelukes, L. Conches, and F. L. Sacerdote, *Biochim. et Biophys. Acta* **26,** 443 (1957).
321a. N. Wolleman and G. Feuer, *Acta Phys. Acad. Sci. Hung.* **7,** 329 (1955); *Chem. Abstr.* **50,** 1097f (1956).
322. P. Eggleton and G. P. Eggleton, *Biochem. J.* **21,** 190 (1927).
323. P. Eggleton and G. P. Eggleton, *J. Physiol.* **65,** 12 (1928).
324. C. H. Fiske and Y. SubbaRow, *Science* **65,** 401 (1927).
325. C. H. Fiske and Y. SubbaRow, *J. Biol. Chem.* **81,** 629 (1929).
326. O. Meyerhof and K. Lohmann, *Biochem. Z.* **196,** 22, 49 (1928).
327. N. v. Thoai and Y. Robin, *Biochim. et Biophys. Acta* **14,** 76 (1953).
328. J. Roche, N. v. Thoai, I. Garcia, and Y. Robin, *Compt. rend. soc. biol.* **146,** 1902 (1952).
329. N. v. Thoai, J. Roche, Y. Robin, and N. v. Thiem, *Compt. rend. soc. biol.* **147,** 1241, 1670 (1953).
330. N. v. Thoai, J. Roche, Y. Robin, and N. v. Thiem, *Biochim. et Biophys. Acta* **11,** 593 (1953).
331. N. v. Thoai and Y. Robin, *Biochim. et Biophys. Acta* **13,** 533 (1954).
332. D. Ackermann, *Z. physiol. Chem.* **299,** 186 (1955); **302,** 80 (1955).
333. G. E. Hobson and K. R. Rees, *Biochem. J.* **61,** 549 (1955).
334. M. V. Tracy, *in* "Modern Methods of Plant Analysis" (K. Paech and M. V. Tracy, eds.), p. 119. Springer, Berlin, 1955.
335. J. R. Porter, "Bacterial Chemistry and Physiology." Wiley, New York, 1946.
336. A. H. Ennor, *in* "Methods in Enzymology" (S. P. Colowick and N. O. Kaplan, eds.), Vol. III, p. 850. Academic Press, New York, 1957.
337. A. H. Ennor and L. A. Stocken, *Biochem. J.* **43,** 190 (1948).

338. J. F. Morrison, A. H. Ennor, and D. E. Griffiths, *Biochem. J.* **68,** 447 (1958).
339. N. v. Thoai and N. v. Thiem, *Bull. soc. chim. biol.* **39,** 355 (1957).
340. D. E. Griffiths, J. F. Morrison, and A. H. Ennor, *Biochem. J.* **65,** 612 (1957).
340a. A. H. Ennor, J. F. Morrison, and H. Rosenberg, *Biochem. J.* **62,** 358 (1956).
340b. I. M. Beatty and D. I. Magrath, *Nature* **183,** 591 (1959).
340c. I. M. Beatty, D. I. Magrath, and A. H. Ennor, *Nature* **183,** 591 (1959).
341. A. H. Ennor and J. F. Morrison, *Physiol. Revs.* **38,** 631 (1958).
342. D. M. Needham, J. Needham, E. Baldwin, and J. Yudkin, *Proc. Roy. Soc.* **B110,** 260 (1932).
342a. E. Baldwin and W. H. Yudkin, *Proc. Roy. Soc.* **B136,** 614 (1949–1950).
342b. E. Baldwin, *Symposia Soc. Exptl. Biol.* **7,** 22 (1953).
343. E. Baldwin and W. H. Yudkin, *Proc. Roy. Soc.* **B136,** 614 (1950).
344. K. Lohmann, *Biochem. Z.* **286,** 28 (1936).
345. A. H. Ennor and H. Rosenberg, *Biochem. J.* **51,** 606 (1952).
345a. H. Rosenberg and A. H. Ennor, *Biochim. et Biophys. Acta* **17,** 261 (1955).
346. H. Barker and A. H. Ennor, *Biochim. et Biophys. Acta* **7,** 272 (1951).
347. G. A. LePage, *in* "Manometric Techniques and Related Methods for the Study of Tissue Metabolism" (W. W. Umbreit, R. H. Burris, and J. F. Stauffer, eds.). Burgess Publishing Co., Minneapolis, 1951.
348. J. Roche, N. v. Thoai, and Y. Robin, *Biochim. et Biophys. Acta* **24,** 514 (1957).
348a. S. Lissitzky, I. Garcia, and J. Roche, *Experientia* **10,** 379 (1954).
349. J. Roche, N. v. Thoai, and J. L. Hatt, *Biochim. et Biophys. Acta* **14,** 71 (1954).
350. P. C. Caldwell, *Biochem. J.* **55,** 458 (1953).
351. T. J. Mende and E. L. Chambers, *Arch. Biochem. Biophys.* **45,** 105 (1953).
352. C. J. Weber, *J. Biol. Chem.* **86,** 217 (1930).
353. D. E. Griffiths, J. F. Morrison, and A. H. Ennor, *Biochem. J.* **65,** 153 (1957).
354. A. H. Ennor, J. F. Morrison, and H. Rosenberg, *Biochem. J.* **62,** 358 (1956).
355. J. Roche, W. Felix, Y. Robin, and N. v. Thoai, *Compt. rend.* **233,** 1688 (1951).
356. N. v. Thoai, *Bull. soc. chim. biol.* **39,** 197 (1957).
356a. O. Cori, A. Traverso-Cori, M. Lagarrigue, and F. Marcus, *Biochem. J.* **70,** 633 (1959).
357. E. Baldwin and J. Needham, *Proc. Roy. Soc.* **B122,** 197 (1937).
358. A. H. Ennor and H. Rosenberg, *Biochem. J.* **56,** 308 (1954).
359. A. H. Ennor and H. Rosenberg, *Biochem. J.* **57,** 203 (1954).
360. I. T. Oliver, *Biochim. et Biophys. Acta* **14,** 587 (1954).
361. J. B. Chappel and S. V. Perry, *Biochem. J.* **57,** 421 (1954).
362. S. A. Kuby, L. Noda, and H. A. Lardy, *J. Biol. Chem.* **209,** 191 (1954).
363. L. Noda, S. A. Kuby, and H. A. Lardy, *J. Biol. Chem.* **209,** 203 (1954).
364. S. A. Kuby, L. Noda, and H. A. Lardy, *J. Biol. Chem.* **210,** 65 (1954).
365. I. Banga, *Studies Inst. Med. Chem. Univ. Szeged* **3,** 59 (1943); *Chem. Abstr.* **41,** 1301 (1947).
365a. J. F. Morrison, A. H. Ennor, and D. E. Griffiths, *Proc. Intern. Symposium on Enzyme Chem., Tokyo and Kyoto, 1957* p. 113 (1958).
366. G. E. Hobson and K. R. Rees, *Biochem. J.* **65,** 305 (1957).
367. E. T. Szorényi, P. D. Dvornikova, and R. G. Degtyar, *Compt. rend. acad. sci. U. R. S. S.* **67,** 341 (1949).
367a. P. Elodi and E. T. Szorényi, *Acta Phys. Acad. Sci. Hung.* **9,** 367 (1956).
368. N. v. Thoai and Y. Robin, *Compt. rend. soc. biol.* **145,** 1674 (1951).

369. N. v. Thoai and Y. Robin, *Compt. rend. sci.* **232,** 452 (1951).
370. G. E. Hobson, *Biochem. J.* **60,** *Proc.,* viii (1955).
370a. O. Meyerhof and H. Green, *J. Biol. Chem.* **183,** 377 (1950).
370b. R. K. Morton, *Nature* **172,** 69 (1953).
370c. R. A. Smith, *J. Am. Chem. Soc.* **81,** 4758 (1959).
371. E. Baldwin, "An Introduction to Comparative Biochemistry," p. 340. Cambridge University Press, London and New York, 1953.
372. M. Florkin, "Biochemical Evolution" (edited, translated, and augmented by S. Morgulis). Academic Press, New York, 1949.
373. L. Hyman, "The Invertebrates," Vol. IV: Echinodermata, p. 700. McGraw-Hill, New York, 1955.
374. I. Garcia and F. Miranda, *Compt. rend. soc. biol.* **148,** 1187 (1954).
374a. J. Roche and Y. Robin, *Compt. rend. soc. biol.* **148,** 1541, 1783 (1954).
375. E. Baldwin and W. H. Yudkin, *Biol. Bull.* **95,** 273 (1948).
376. I. Greenwald, *J. Biol. Chem.* **162,** 239 (1946).
376a. J. F. Morrison, D. E. Griffiths, and A. H. Ennor, *Nature* **178,** 359 (1956).
376b. P. Eggleton and G. P. Eggleton, *J. Physiol.* **65,** 15 (1928).
377. F. Lynen, E. Reichert, and L. Rueff, *Ann.* **574,** 1 (1951); *Z. angew. Chem.* **63,** 490 (1951); *Federation Proc.* **12,** 683 (1953).
378. F. Lipmann, *J. Biol. Chem.* **160,** 173 (1945).
379. D. Nachmansohn and A. L. Machado, *J. Neurophysiol.* **6,** 397 (1943).
380. Symposium on The Chemistry and Functions of Coenzyme A, *Federation Proc.* **12,** 673 (1953).
381. F. Lipmann, *Science* **120,** 855 (1954).
382. A. H. Mehler, "Introduction to Enzymology," p. 85. Academic Press, New York, 1957.
383. J. Baddiley, *Advances in Enzymol.* **16,** 1 (1955).
383a. G. J. Moffatt and H. G. Khorana, *J. Am. Chem. Soc.* **81,** 1265 (1959).
384. E. J. Simon and D. Shemin, *J. Am. Chem. Soc.* **75,** 2520 (1953).
385. T. Wieland and L. Rueff, *Z. angew. Chem.* **65,** 186 (1953).
386. T. Wieland and H. Köppe, *Ann.* **581,** 1 (1953); **588,** 15 (1954).
387. H. Beinert, *Federation Proc.* **12,** 681 (1953).
388. H. A. Barker, E. R. Stadtman, and A. Kornberg, *in* "Methods in Enzymology" (S. P. Colowick and N. O. Kaplan, eds.), Vol. I, p. 599. Academic Press, New York, 1955.
389. E. R. Stadtman, *in* "Methods in Enzymology" (S. P. Colowick and N. O. Kaplan, eds.), Vol. III, p. 931. Academic Press, New York, 1957.
390. R. R. Grunert and P. H. Phillips, *Arch. Biochem.* **30,** 217 (1951).
391. P. D. Boyer, *J. Am. Chem. Soc.* **76,** 4331 (1954).
392. E. R. Stadtman, M. Doudoroff, and F. Lipmann, *J. Biol. Chem.* **191,** 377 (1951).
393. J. R. Stern, M. J. Coon, A. del Campillo, *J. Biol. Chem.* **221,** 1 (1956); *Nature* **171,** 28 (1953).
394. D. E. Green, D. S. Goldman, S. Mii, and H. Beinert, *J. Biol. Chem.* **202,** 137 (1953).
395. F. Lynen, L. Wessely, O. Wieland, and L. Rueff, *Z. angew. Chem.* **64,** 687 (1952).
396. J. R. Stern, M. J. Coon, and A. del Campillo, *J. Am. Chem. Soc.* **75,** 1517 (1953).
397. M. Katagiri and O. Hayaishi, *Federation Proc.* **15,** 285 (1956).

398. W. G. Robinson, B. K. Bachhawat, and M. J. Coon, *J. Biol. Chem.* **218,** 391 (1956).
398a. O. Hayaishi, *J. Am. Chem. Soc.* **75,** 4367 (1953).
398b. J. B. Wolfe, D. Ivler, and S. C. Rittenberg, *J. Biol. Chem.* **209,** 867 (1954).
399. R. M. Burton and E. R. Stadtman, *J. Biol. Chem.* **202,** 873 (1953).
400. S. Ochoa, J. R. Stern, and M. C. Schneider, *J. Biol. Chem.* **193,** 691 (1951).
401. I. C. Gunsalus, *in* "Mechanism of Enzyme Action" (W. D. McElroy and B. Glass, eds)., p. 545. Johns Hopkins, Baltimore, 1954.
401a. W. B. Jakoby and E. W. Yamada, *Biochim. et Biophys. Acta* **34,** 276 (1959).
402. M. Flavin and S. Ochoa, *J. Biol. Chem.* **229,** 965 (1957).
403. D. R. Sanadi and J. W. Littlefield, *J. Biol. Chem.* **201,** 103 (1953).
404. E. R. Stadtman, *J. Biol. Chem.* **203,** 501 (1953).
405. R. O. Brady and E. R. Stadtman, *J. Biol. Chem.* **211,** 621 (1954).
406. E. Racker, *Federation Proc.* **12,** 711 (1953).
407. F. M. Huennekens, *in* "Currents in Biochemistry" (D. E. Green, ed.), p. 493. Interscience, New York, 1956.
408. D. E. Green, *Biol. Revs. Cambridge Phil. Soc.* **29,** 330 (1954).
409. J. R. Stern, A. del Campillo, and I. Raw, *J. Biol. Chem.* **218,** 971 (1956).
410. R. C. Williams, C. M. Lyman, G. E. Goodyear, J. H. Truesdail, and D. Holaday, *J. Am. Chem. Soc.* **55,** 2912 (1933).
411. G. D. Novelli, *Physiol Revs.* **33,** 525 (1953).
412. E. R. Stadtman and F. H. White, *J. Am. Chem. Soc.* **75,** 2022 (1953).
413. J. H. Boyer, *J. Am. Chem. Soc.* **74,** 6274 (1952).
414. T. Wieland and G. Schneider, *Ann.* **580,** 159 (1953).
415. J. Baddiley, J. G. Buchanan, and R. Letters, *J. Chem. Soc.* p. 2812 (1956).
415a. W. P. Jencks and J. Carriuolo, *J. Biol. Chem.* **234,** 1272, 1280 (1959).
416. M. L. Bender and B. W. Turnquest, *J. Am. Chem. Soc.* **79,** 1652, 1656 (1957).
417. T. C. Bruice and G. L. Schmir, *J. Am. Chem. Soc.* **79,** 1663 (1957).
418. G. H. Dixon and H. Neurath, *J. Biol. Chem.* **225,** 1049 (1957).
419. C. E. McDonald and A. K. Balls, *J. Biol. Chem.* **227,** 727 (1957).
420. L. W. Cunningham, *Science* **125,** 1145 (1957).
421. D. J. Bauer, *in* "The Nature of Virus Multiplication" (Symp. Soc. Gen. Microbiol.), p. 46. Cambridge University Press, London and New York, 1953.
422. W. O. James, *Advances in Enzymol.* **18,** 281 (1957).
423. W. Vishniac, B. L. Horecker, and S. Ochoa, *Advances in Enzymol.* **19,** 1 (1958).
424. R. G. Owens, *Science* **122,** 415 (1955).
425. J. Brachet, *Biochim. et Biophys. Acta* **18,** 247 (1955).
426. G. R. Seaman, *J. Biol. Chem.* **228,** 149 (1957).
427. K. Lohmann, *Biochem. Z.* **203,** 164 (1928).
428. G. M. Price and S. E. Lewis, *Biochem. J.* **68,** 11p (1958).
429. A. H. Whiteley, *Am. Naturalist* **83,** 249 (1949).
429a. H. F. Blum, "Time's Arrow and Evolution." Princeton Univ. Press, Princeton, New Jersey, 1951.
430. A. Gulick, *Am. Scientist* **43,** 479 (1955).
431. H. F. Blum, *in* "Rhythmic and Synthetic Processes in Growth" (D. Rudnick, ed.), p. 155. Princeton University Press, Princeton, N. J., 1957.
432. H. F. Blum, *Am. Scientist* **43,** 595 (1955).

433. J. W. S. Pringle, *Symposia Soc. Exptl. Biol.* **7,** 1 (1953).
433a. J. M. Lowenstein, *Biochem. J.* **70,** 222 (1958).
433b. M. Calvin, *Science* **130,** 1170 (1959).
434. F. Kutscher and D. Ackermann, *Z. Biol.* **84,** 181 (1926).
435. N. J. Berrill, "The Origin of Vertebrates." Oxford Univ. Press, London and New York, 1955.
436. G. Wald, *in* "Trends in Physiology and Biochemistry" (E. S. G. Barron, ed.), p. 337. Academic Press, New York, 1952.

CHAPTER 5

Onium Compounds and Their Biological Significance

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I. General Introduction, Natural Distribution, and Chemical Properties of Onium Compounds

Onium compounds* are widely but unevenly distributed in nature. They occur most abundantly and in the greatest variety in marine invertebrates, fishes, and certain plants, while in microorganisms and in vertebrates only a few compounds are found and in relatively small amounts.

* Onium compounds are addition compounds to which "a definite structure can be ascribed only on the basis of an extension of the simple valence theory." More precisely they may be defined as "substances formed by an addition reaction, in the course of which some atom increases its valence by one unit and, in doing so, increases its formal charge algebraically by one unit" (*1*).

TABLE I

NATURAL OCCURRENCE AND DISTRIBUTION OF ONIUM COMPOUNDS

	Micro-organisms	Plants	Inverte-brates	Verte-brates	References
I. Aliphatic quaternary ammonium compounds					
1. Tetramethyl ammonium hydroxide		+	+		*a*
2. Neurine (trimethylvinyl ammonium hydroxide)	+			+	*a*
3. Choline	+	+	+	+	*b*
4. Betaine	+		+	+	*a,c*
5. Muscarine		+			*d*
6. Homobetaine (β-propiobetaine)			+		*a*
7. γ-Butyrobetaine			+	+	*e,f*
8. Crotonobetaine				+	*a*
9. Carnitine (β-hydroxybutyrobetaine)	+		+	+	*e,g*
10. Miokinine				+	*h*
11. Asterubin			+		*a*
12. Ercinine		+	+		*i*
13. Ergothioneine	+	+		+	*j*
14. Hypaphorine		+			*a,k*
15. Bufotenidine		+		+	*l,k*
16. Leptodactyline				+	*m*
17. Candicine		+	+		*k*
18. Coryneine		+	+		*k*
II. Heterocylic onium compounds					
1. Stachidrine		+			*n,o*
2. Betonicine		+			*a*
3. Homostachidrine		+			*o*
4. Atisine		+			*p*
5. Thiamine	+	+	+	+	*q*
6. DPN (TPN)	+	+	+	+	*r*
7. *N'*-Methylnicotinamide		+		+	*s*
8. *N'*-Methylpicolinic acid			+	+	*t*
9. *N'*-Methylpyridine				+	*u*
10. Curare alkaloids		+			*v*
III. Sulfonium compounds					
1. Dimethylpropiothetin		+			*w*
2. *S*-Adenosylmethionine	+			+	*x*
3. *S*-Methylmethionine		+			*y,z*

[a] Guggenheim (*11*). [b] Best *et al.* (*37*). [c] Cromwell and Rennie (*53*). [d] F. Kögl, H. Duisberg, and H. Erxleben, *Ann.* **156,** 489 (1931). [e] Carter *et al.* (*59*). [f] Hosein and McLennan (*64*). [g] Kutscher (*58*). [h] Ackermann (*29*). [i] Kutscher (*83*); Reuter (*84*). [j] See references *65-75*. [k] L. Reti, *in* "The Alkaloids" (R. H. F. Manske and H. L.

Information as to the biological significance of these compounds, their mode of synthesis, and the role which they fulfill in the various species in which they are found is extremely uneven. Thus practically nothing is known about the biosynthesis of carnitine and its function in muscle or about the role, origin, and fate of the betaines and the thetins in the plants in which they are found. On the other hand considerable information has accumulated on the biochemistry of certain other sulfonium compounds and their participation in biological transalkylation reactions. Likewise the biological and biochemical role of certain thiazolium and pyridinium compounds is relatively fully understood and is closely related to the well known coenzyme functions of thiamine pyrophosphate and of the pyridine nucleotides.

Largely as the result of work on enzymatic mechanisms involved in transalkylation reactions from sulfonium or quaternary ammonium compounds and in base exchange reactions utilizing thiazolium or pyridinium compounds, the concept has emerged in recent years that onium compounds are energy-rich in the same sense in which certain pyrophosphate or acyl phosphate compounds and acyl mercaptan compounds are energy-rich, namely, they can serve biologically as sources of chemical energy for biosynthetic purposes. This concept will be discussed in detail in a later section but must be anticipated now for it will be referred to throughout the text and it is the main *raison d'être* for this chapter.

Finally, it should be pointed out that some onium compounds are exceedingly active biologically and in some cases are among the most potent pharmacological agents known. Little or no knowledge is available, however, of the intimate mechanism by which they exert these effects.

It is clearly beyond the scope and limits of this chapter to review fully all aspects of the chemistry and biology of naturally occurring onium compounds. This review will attempt to present an over-all view of the natural distribution and the significant chemical properties of onium compounds and attempt a more complete discussion of those

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Holmes, eds.), Vol. 3, p. 313. Academic Press, New York, 1953. [l] V. Erspamer, *Rend. sci. farmitalia* **1,** 1 (1954). [m] V. Erspamer, personal communication (1900). [n] Jahkns (*93*). [o] Wichler and Marion (*94*). [p] E. S. Stern, *in* "The Alkaloids" (R. H. F. Manske and H. L. Holmes, eds.), Vol. 4, p. 275. Academic Press, New York, 1954. [q] C. P. Jansen, *in* "The Vitamins" (W. H. Sebrell, Jr., and R. S. Harris, eds.), Vol. 3, p. 472. Academic Press, New York, 1954. [r] F. Schlenk, *in* "The Enzymes" (J. B. Sumner and K. Myrbäck, eds.), Vol. 2, Part 1, p. 250. Academic Press, New York, 1951. [s] Perlzweig *et al.* (*101*). [t] Kutscher and Ackermann (*97*); Gasteiger *et al.* (*99*). [u] Cantoni (*23*). [v] L. E. Craig, *Chem. Revs.* **42,** 285 (1948). [w] Challenger and Simpson (*109*). [x] See references *122–127*. [y] McRorie *et al.* (*151*). [z] Challenger and Hayward (*153*).

relatively few compounds about which more is known from the point of view of enzymology and/or intermediary metabolism.

A. Natural Distribution of Onium Compounds

Listed in Table I are a variety of naturally occurring compounds containing an onium pole. Although the list does not claim to be a complete one, an attempt has been made to include most of the known compounds where data are available. The distribution of the compounds is also given in the table. In some cases, although the compound itself may not have been isolated from natural sources, the enzymatic equipment for its formation has been identified, and it may be presumed then that under suitable conditions of nutrition or of extraction the compound itself might be found. Chemical formulas are given in Table II.

B. Considerations on the Chemistry of Onium Compounds*

The presence of an onium center renders a molecule susceptible to the nucleophilic attack which is characteristic of compounds of this class. These reactions may be divided broadly into three groups depending on the point of attack of the nucleophilic reagent.

In the first class there is an attack at the α-carbon atom on the onium pole with heterolytic elimination of the latter center as an uncharged molecule. A simple example is the decomposition of a trimethylsulfonium salt (Eq. 1).

$$Y + \begin{matrix} CH_3 \\ \\ CH_3 \end{matrix}\!\!>\!\overset{\oplus}{S}\!-\!CH_3 \rightarrow CH_3\!-\!Y + CH_3\!-\!S\!-\!CH_3 \qquad (1)$$

With strongly nucleophilic reagents, such as hydroxide and phenoxide ion this reaction will proceed by the bimolecular (S_N2) mechanism, while with those of weaker nucleophilic power such as acetate and chloride the rate is independent of the attacking reagent and proceeds by the unimolecular (S_N1) mechanism. In an unsymmetrically substituted onium compound, nucleophilic attack by Y is facilitated by substituents which cause electron removal from the α-carbon atom attacked, while the same effect will reduce the tendency of the hetero atom to separate with its bonding electrons. Thus the effect of substituents on the bimolecular mechanism is liable to be small and indeterminate while in the S_N1 mechanism the rate-determining step will be facilitated by electron accession to the α-carbon atom.

A number of well-defined enzymatic reactions to be discussed later

* The author is particularly indebted to Dr. Graham Jamieson for his valuable help in the preparation of this section.

TABLE II

CHEMICAL STRUCTURE OF ONIUM COMPOUNDS

I. ALIPHATIC QUATERNARY AMMONIUM COMPOUNDS

1. $[(CH_3)_4\overset{\oplus}{N}]OH$

2. $(CH_3)_3\overset{\oplus}{N}-CH=CH_2$

3. $(CH_3)_3\overset{\oplus}{N}-CH_2-CH_2-OH$

4. $(CH_3)_3\overset{\oplus}{N}-CH_2-CO_2^-$

5. $(CH_3)_3\overset{\oplus}{N}-CH(CH_2-CHO)(CHOH-CH_2-CH_3)$

6. $(CH_3)_3\overset{\oplus}{N}-CH_2-CH_2-CO_2^-$

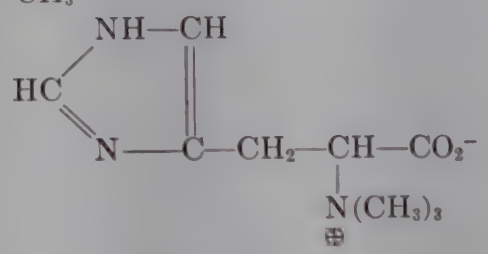
7. $(CH_3)_3\overset{\oplus}{N}-CH_2-CH_2-CH_2-CO_2^-$

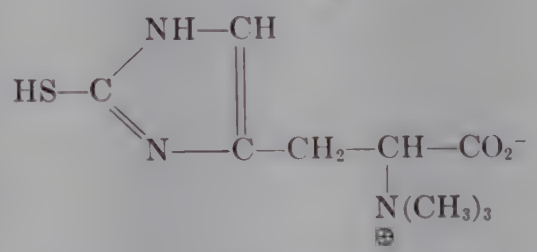
8. $(CH_3)_3\overset{\oplus}{N}-CH_2-CH=CH-CO_2^-$

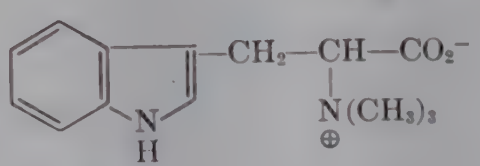
9. $(CH_3)_3\overset{\oplus}{N}-CH_2-CHOH-CH_2-CO_2^-$

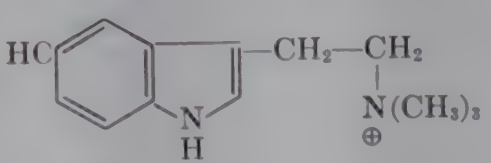
10. $(CH_3)_3\overset{\oplus}{N}-CH_2-CH_2-CH_2-CH(N(CH_3)_3^{\oplus})-CO_2^-$

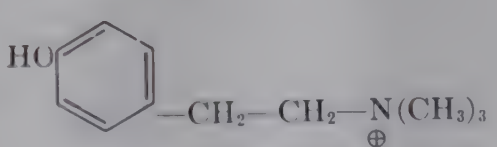
11. $H-\overset{\oplus}{N}(CH_3)(CH_3)-C(=NH)-N(H)-CH_2-CH_2-CH_2-SO_3^-$

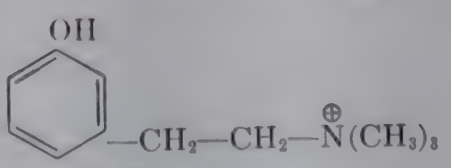
12. 

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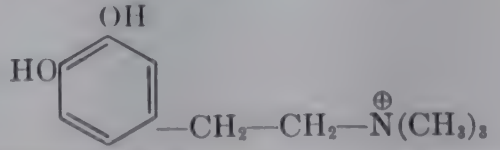
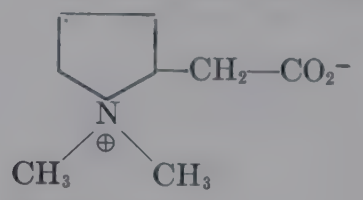
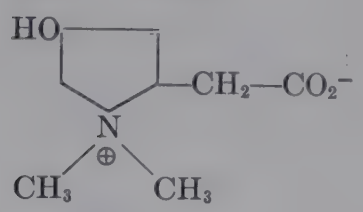
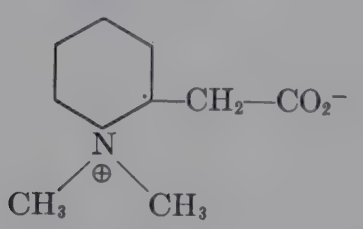
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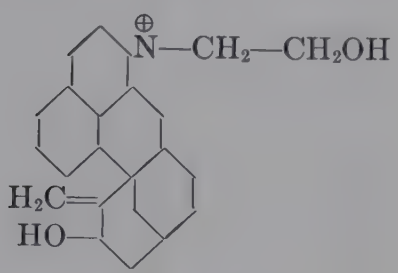
TABLE II (*Continued*)

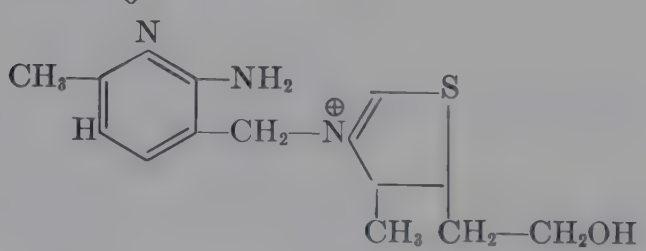
II. HETEROCYLIC ONIUM COMPOUNDS

1.


2.


3.


4.


5.


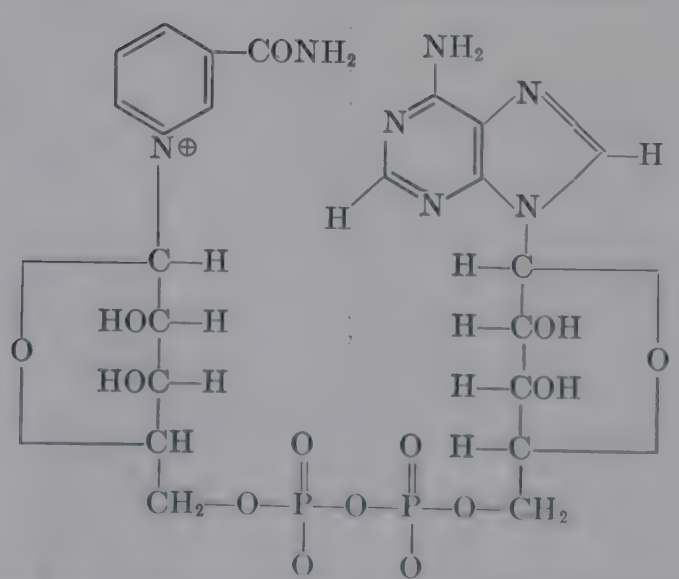
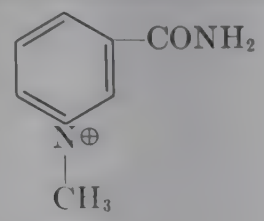
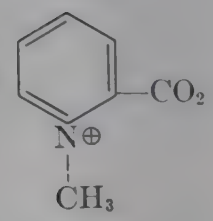
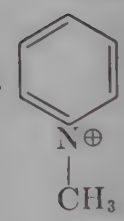
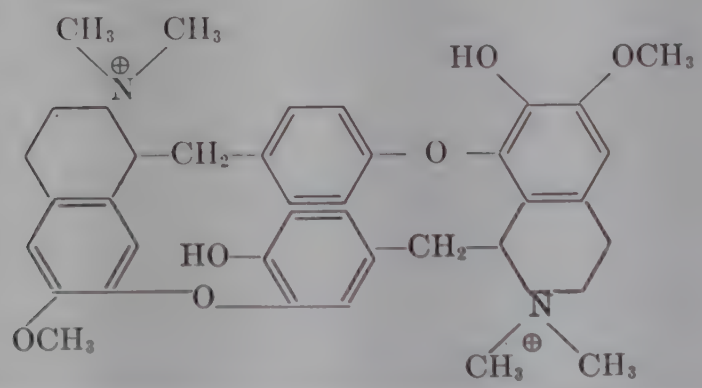
6.


TABLE II (*Continued*)

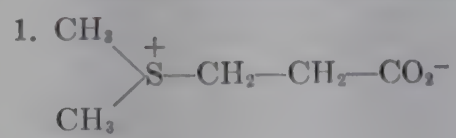
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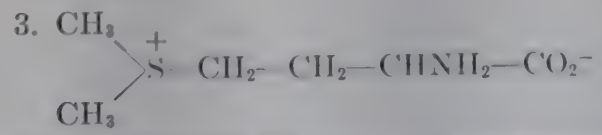
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10. 

III. SULFONIUM COMPOUNDS

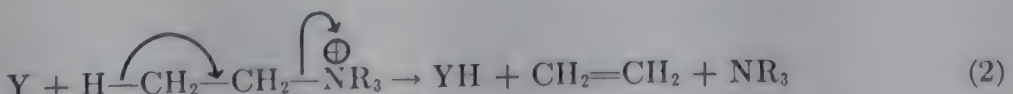
1. 

2. See Fig. 6.

3. 

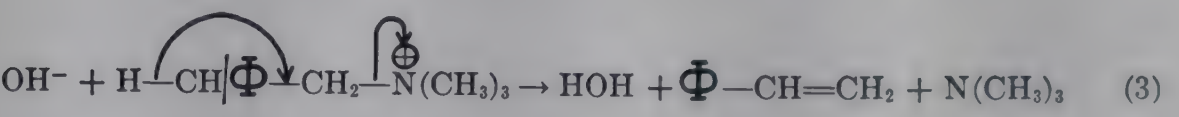
may be included in this class, among them those involved in the biosynthesis of creatine, 3-methoxyepinephrine, and spermidine.

In the second class elimination results from nucleophilic attack on a β-hydrogen with expulsion of the hetero atom. The reaction may be generally formulated as in Eq. 2:



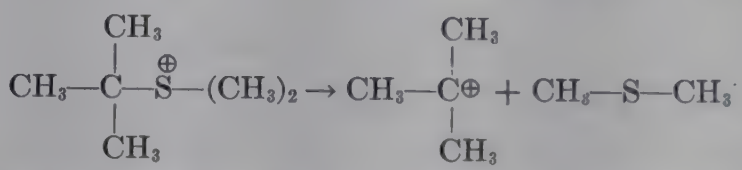
$$Y + H{-}CH_2{-}CH_2{-}\overset{\oplus}{N}R_3 \rightarrow YH + CH_2{=}CH_2 + NR_3 \qquad (2)$$

As might be expected the reaction can proceed simultaneously by both of the above mechanisms, the relative preponderance of substitution over elimination depending on the relationship between the nucleophilic nature of the attacking group (its affinity for an atomic nucleus) and its basicity (affinity for a proton). With a strongly basic nucleophilic reagent, such as hydroxide ion, elimination might be expected to proceed by a bimolecular (E2) reaction, as in thermal decomposition of quaternary ammonium hydroxides (Eq. 3), and to be favored by substituents which will promote electron secession from the β-carbon.



$$OH^- + H{-}CH(\Phi){-}CH_2{-}\overset{\oplus}{N}(CH_3)_3 \rightarrow HOH + \Phi{-}CH{=}CH_2 + N(CH_3)_3 \qquad (3)$$

With reagents of low nucleophilic character, as at low pH values (i.e., extremely low concentrations of hydroxide ion) or in solution of high dielectric constant, reaction can proceed by a unimolecular mechanism (E1) in which the carbonium ion formed in the rate-determining step (Eq. 4) is identical for both the elimination and substitution reac-



$$CH_3{-}\overset{\displaystyle CH_3}{\underset{\displaystyle CH_3}{C}}{-}\overset{\oplus}{S}{-}(CH_3)_2 \rightarrow CH_3{-}\overset{\displaystyle CH_3}{\underset{\displaystyle CH_3}{C^{\oplus}}} + CH_3{-}S{-}CH_3 \qquad (4)$$

tions. Hence the relative amounts of products formed by these two mechanisms will be substantially independent of the nature of the onium group.

The mechanisms of these reactions have been deduced from extensive and masterly studies by Ingold and his collaborators to whose publications the reader is referred for a detailed exposition of the influence of structural and environmental factors on the course of onium substitution and elimination reactions (2–4).

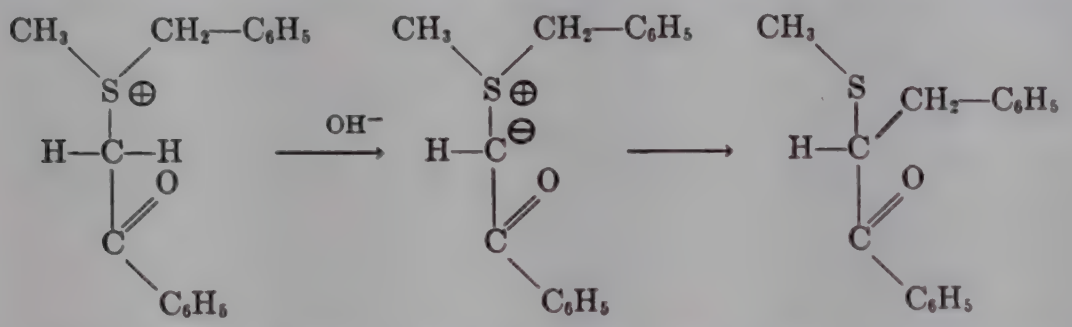
One example of a biological elimination reaction is the enzymatic conversion of dimethylpropiothetin to acrylic acid; this and other similar reactions will be discussed later and it will also be shown that such mechanisms can be used to explain the occurrence of a number of other compounds. Since substitution reactions occur enzymatically with nucleophilic reagents which might be expected to cause a certain amount of 1 : 2 elimination it seems plausible to suggest that at least part of the function of the enzyme is to direct the reagent exclusively towards α-substitution.

The third class of reactions is different insofar as it involves an *intramolecular* migration to an anionoid center. Probably the simplest and best known example is the so-called "Willstätter Migration" (5).

Although this reaction (Eq. 5) has not been demonstrated biologically, its existence would provide a facile explanation for the mechanism

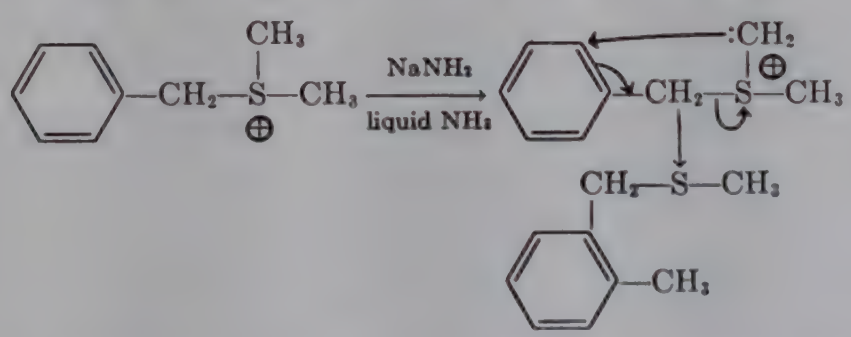
$$(CH_3)_3\overset{\oplus}{N}-CH_2-COO^- \rightarrow (CH_3)_2-N-CH_2-COO-CH_3 \qquad (5)$$

of biosynthesis of certain compounds. Furthermore, under strongly basic conditions certain onium compounds will rearrange via an intermediate carbanion. The Stevens rearrangement is formulated as in Reaction 6 on the basis of the alkaline dependence of the reaction, its established intramolecular nature and the structural requirements



(6)

for reactivity (*6*). This 1:2 rearrangement has been realized with both quaternary nitrogen (*6*) and sulfonium (*7*) compounds. A related migration of benzyl-alkyl onium compounds, under the influence of lithium or sodium amide in liquid ammonia to effect *ortho* methyl substitution in the aromatic ring (Eq. 7) is also known (*8*).



(7)

Although the biological implication of these carbanion rearrangements is as yet undetermined they may be of significance in the biosynthesis of nuclear methyl groups [e.g., in mycophenolic acid, see Birch *et al.* (*9*)] derived from methionine.

II. Aliphatic Quaternary Ammonium Compounds

The structural similarities of betaine, homobetaine (β-propiobetaine), carnitine, miokinine, ercinine, hypaphorine, bufotenidine, candicine, stachydrine, and methionine methyl sulfonium and the amino acids glycine, β-alanine, γ-aminobutyric acid, ornithine, histidine, tryptophan, tyrosine, and methionine (or the corresponding amines) are obvious.

Prima facie the similarity between glycine and betaine, tryptophan and hypaphorine, histidine and ercinine, ornithine and miokinine suggests that the biosynthesis of these quaternary onium compounds might involve one of two alternative schemes: (*a*) consecutive methylation of the amino acid with all three methyl groups being supplied *as such* from a methyl donor compound (*10*); or (*b*) a mechanism involving *de novo* synthesis of the methylated compound from one-carbon precursors and the parent compound.

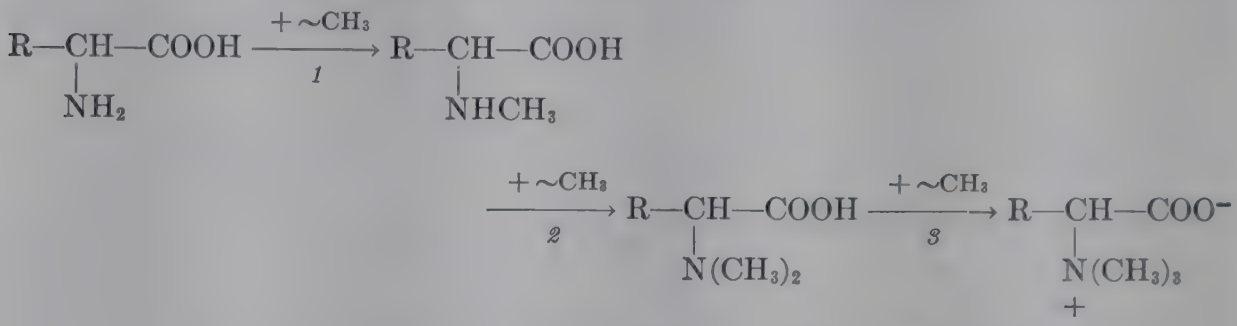


FIG. 1. Formation of betaines by consecutive methylation.

The first scheme, outlined diagrammatically in Fig. 1, requires that *N*-dimethylamino acids should be intermediates in the biosynthetic pathway. It must be pointed out, however, that *N*-dimethylamino acids are not known to exist biologically, whereas methyl and dimethyl amines are. Nevertheless it is likely that the biosynthesis of ergothioneine (see Section II,A,4) follows this pattern and the same might be possible for the formation of tetramethylammonium hydroxide, bufotenidine, and candicine, about which unfortunately nothing is known.

On the other hand, what is known about the biosynthesis of betaine does not fit scheme 1, inasmuch as betaine is not formed biologically by the consecutive methylation of glycine, but by a two-step oxidation of choline through the corresponding aldehyde (Fig. 2, steps 5 and 6) (*11, 12, 13*). It is now well established that in vertebrates (*14*), as well as in microorganisms and higher plants, choline itself is derived from aminoethanol, the product of serine decarboxylation (Fig. 2, step 1), by a series of steps involving both *de novo* synthesis of a methyl group from one-carbon precursors (Fig. 2, steps 2 and 3) (*15, 16*) and transfer of a methyl group from a methyl donor compound (Fig. 2, step 4) (*17*).

Since the biochemical reactions involving transformation of ethanolalamine into choline are known to occur widely and must therefore be considered to be a major biochemical pathway, it is attractive from the standpoint of comparative biochemistry and of biochemical evolution to postulate that the synthesis of other betaines might proceed by a series of reactions similar to those shown in Fig. 2. This might be pos-

sible for the biosynthesis of homobetaine (from homoserine → β-propanolamine → homocholine) and of ercinine from the corresponding amino alcohol, histidinol, which has been reported as an intermediate in the pathway of histidine biosynthesis (*18, 19*); scheme 2 might also account for the synthesis of hypaphorine.* The essential differences between the two biosynthetic pathways, shown in Figs. 1 and 2 respectively, lie in the nature of the precursors of the quaternary onium compounds (amino acids or amino alcohols) and in the origin of the

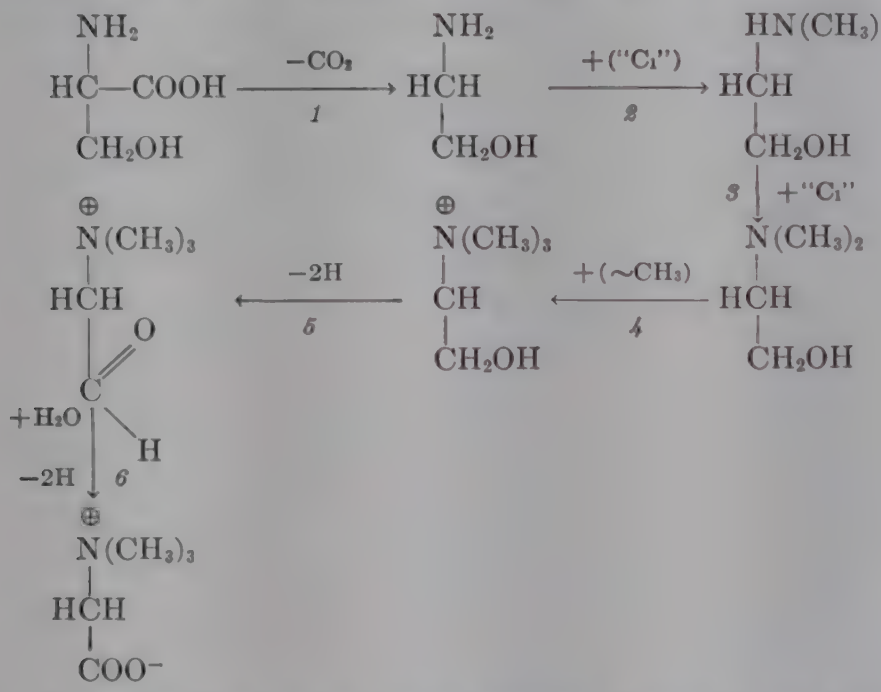


FIG. 2. The biosynthesis of choline, betaine aldehyde, and betaine.

methyl group (transmethylation or *de novo* synthesis of methyl groups plus transmethylation). Moreover, in the formation of a betaine from the corresponding fully methylated amino alcohol an energy-yielding oxidation sequence is required and this may be significant in the overall cell economy, since as shall become clearer later on, transmethylation reactions are energy-requiring processes.

Scheme 2, however, like scheme 1, does not adequately account for the formation of carnitine and a number of other quaternary ammonium compounds. Further alternatives therefore must be considered.

Nothing is known about the biosynthesis of carnitine. It has been suggested before (*22, 23*) that choline aldehyde might be an intermediate and this hypothesis, although totally lacking experimental support, still appears attractive because it utilizes a series of steps fully

* Indole aminoethanol has not been implicated in the biosynthesis of tryptophan but it might arise from indole-3-glycerol phosphate (*20*) in a series of reactions entirely analogous to the ones leading to formation of histidinol from imidazoleglycerol phosphate (*21*).

analogous to known biochemical reactions. The proposed, and still hypothetical reaction sequence is detailed in Fig. 3, and it can be seen that this scheme would also result in the formation of crotonobetaine and butyrobetaine. The condensation of choline aldehyde with acetyl-CoA (step 1) is entirely analogous, biochemically, to the condensation

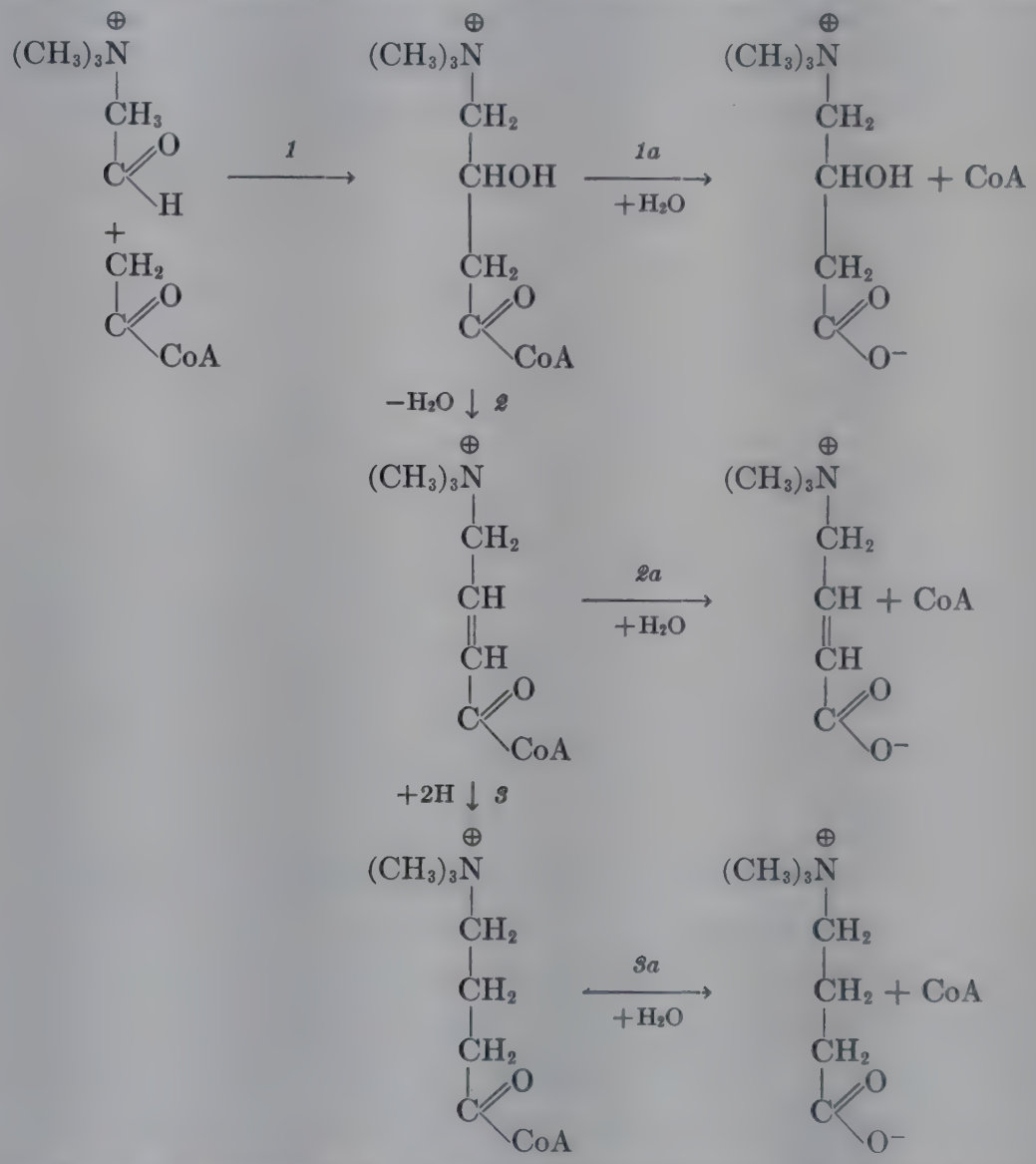


FIG. 3. The biosynthesis of carnitine, crotonobetaine, and γ-butyrobetaine.

of acetyl-CoA with oxaloacetate to yield citric acid (*24*). The dehydration to crotonobetainyl-CoA and the reduction to γ-butyrobetainyl-CoA (steps 2 and 3) are formally identical to the reactions catalyzed respectively by the enzymes crotonase (*25*) and β-hydroxybutyric dehydrogenase (*26, 27*) in the fatty acid cycle. Likewise removal of the CoA moiety could be by a deacylase reaction, for which there is good biochemical precedent (*28*).*

* *Note added in proof*: Evidence in support of this biosynthetic scheme is supplied by the recent finding of Hosein (*28a*) that γ-butyrobetaine can be found in normal mammalian brain as the CoA derivative.

Scheme 3 can be modified to account for the biosynthesis of miokinine (29) as shown in Fig. 4. Steps 1 and 2 are completely analogous to steps 1 and 2 in Fig. 3. Step 3 has not been encountered before but, as will be evident from the earlier discussion, it may be considered as the

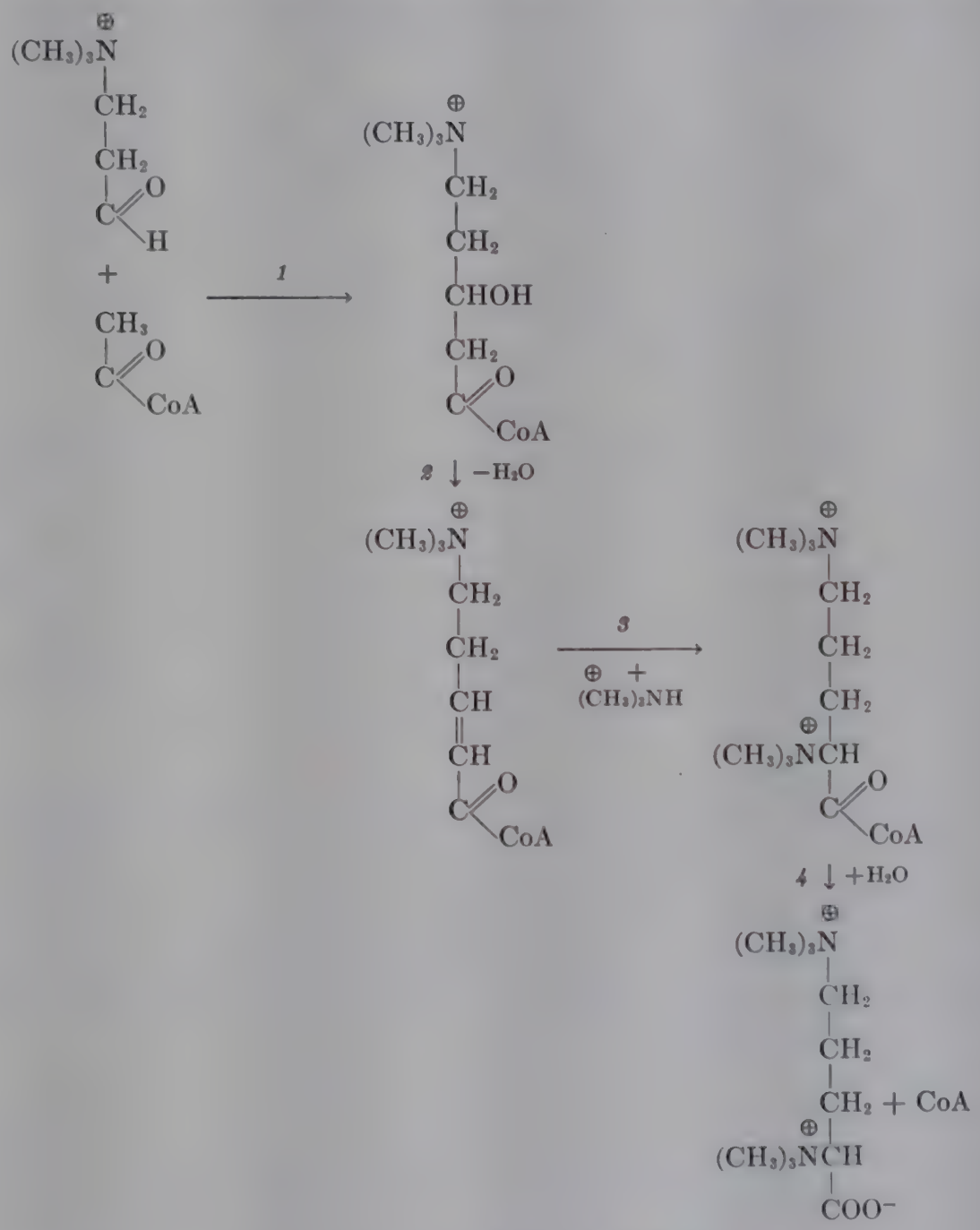


FIG. 4. The biosynthesis of miokinine.

reverse of an elimination reaction from an onium pole, and is quite possible both on chemical and biochemical grounds. Moreover it has some analogy to the biosynthesis of β-alanine from NH_3 and propionyl-CoA, a reaction recently studied by Stadtman (30). The biological utilization of trimethylamine would be interesting since trimethylamine is formed from choline both by aerobic and anaerobic bacteria and by a

variety of plants (*31*), where its formation is greatest during the period of active vegetative growth. In plants, in aerobic bacteria such as *Psuedomonas,* and in other gram-negative organisms (*32, 33*) trimethylamine may be derived from choline by an elimination reaction formally analogous to the formation of dimethyl sulfide from the sulfonium analog of homobetaine (*34*) (see Section IV,A,1). In anaerobes on the other hand (*35*) Hayward and Stadtman have observed the reaction shown in Eq. 8 in which it would appear as if a dismutation of 2 moles of choline aldehyde is synchronous with cleavage of the onium bond.

$$H_2O + 2\ \text{Choline} \rightarrow 2(CH_3)_3N + CH_3{-}CH_2OH + CH_3COOH + 2H^+ \qquad (8)$$

This reaction may account also for the earlier observations of Dyer and Wood (*36*) who found that organic acid was formed in the bacterial metabolism of choline.

It must of course be quite clear by now that the proposed biogenetic schemes are rich in speculation and short in evidence. The fundamental assumption underlying the various hypothetical pathways discussed in schemes 1, 2, 3, and 4, however, is widely accepted and indeed forms one of the cornerstones of comparative biochemistry. This assumption postulates that the living cell utilizes for its biosynthetic purposes only a relatively small number of reactions, and modifies them according to its special requirements along a pattern that, in music, is known as *Theme and Variations.*

A. Biochemistry of Aliphatic Quaternary Ammonium Compounds

As pointed out above, knowledge of the biochemistry of aliphatic quaternary ammonium compounds is sketchy. In some cases, choline, for instance, an enormous literature has accumulated. In most other cases, however, little is known beyond the structure of the compound and one or more sources of origin.

1. *Choline*

Choline is probably the onium compound with the widest distribution as it is found in almost all living cells, predominantly or perhaps exclusively in combined form mainly in the phospholipid fraction. The subject has been recently and repeatedly reviewed (*37*) and only a few comments need to be made at this time.

Quantitatively the role of choline as a constituent of the phospholipids of the lecithin and sphingosine type overshadows other possible functions of choline. Qualitatively, however, choline esters in minute amounts play a fundamental role in neurophysiology (*38, 39*). Although acetylcholine is the best known member of this family it is of interest,

from the point of view of comparative biochemistry, to indicate that a number of other esters of choline have been identified in biological material and found to be extremely active physiologically. Thus Erspamer identified urocanylcholine (*40*) as the active agent in murexine and Whittaker isolated propionylcholine from mammalian spleen (*41*) and β,β-dimethylacrylylcholine from *Thais floridiana* (*42*), the Southern oyster drill.

Choline esters exhibit extraordinary pharmacological activity and elicit a kaleidoscopic variety of physiological responses which span the extremes of stimulation and inhibition. Powerful and specific enzymes regulate their biosynthesis and, more significantly, their degradation. Because of their biological lability, the natural existence of biologically

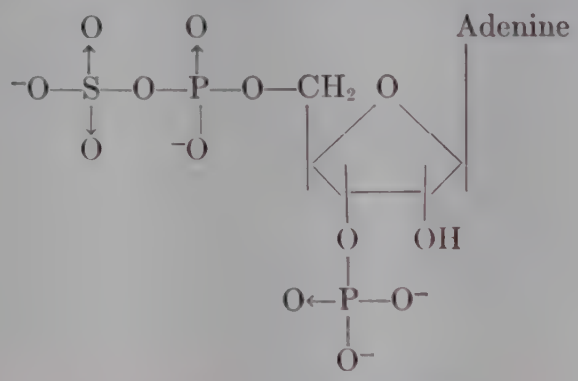


FIG. 5. Phosphoadenylosulfate.

active choline esters was discovered only *after* their physiological role as neurohormones had been invoked. In the light of recent discoveries, there is every reason to believe that there may exist in nature a number of yet undiscovered choline esters which might be endowed with powerful and significant physiological activity.

The biological acetylation of choline follows the pattern of other acetylation reactions and depends on the enzymatic transfer of the acetyl group of acetyl-CoA to choline (*43*). It may be presumed that urocanylcholine, propionylcholine, β,β-dimethylacrylylcholine and certain other choline esters are formed through an analogous series of reactions in which, after activation of the different carboxylic acids to the corresponding CoA derivatives, the acyl moiety are transferred to choline by specific enzymes. On the other hand choline sulfate, another ester of choline with wide natural distribution, is probably formed by a different mechanism. Although no precise information as to its biosynthesis is available it may be postulated that choline sulfate is formed biologically by the reaction of choline with phosphoadenylosulfate, Fig. 5, a compound recently discovered by Robbins and Lipmann (*44*) during their studies on sulfate activation and considered as the key inter-

mediate in biological sulfurylations (*45*).* Choline sulfate was first found by Woolley and Peterson in cultures of *Penicillium* (*46*) and more recently it has been described in a wide variety of other fungi (*47, 48*) and in sea weeds (*49*).

2. *Betaine and Homobetaine*

Betaine is widely distributed in a variety of plant tissues and occurs in relatively high concentration in some species of Leguminosae and Graminacae and in evergreens. In these plants it is found in all tissues but is in highest concentration in the leaves, where it accumulates, especially in older plants. Table III, derived from a paper of Cromwell and

TABLE III

THE BETAINE CONTENT OF TISSUES OF *Beta vulgaris*, L., DURING GROWTH[a]

Age (Days)	Betaine content[b]	
	Leaf	Root
25	21.3	108.5
33	30.7	—
62	71.8	100.0
132	160.0	67.5
132	Outer 218.0	15.4
132	Middle 286.0	—
132	Inner 414.0	—

[a] From data by B. T. Cromwell and S. D. Rennie, *Biochem. J.* **55,** 189 (1953).

[b] Micromoles per gram wet weight.

Rennie (*50*), shows the relationship between age and betaine distribution in the leaves and root of *Beta vulgaris*. It can be clearly seen that the betaine content in the leaves increases with age, while it decreases in the root system. Other data show that a concentration gradient of betaine is established during vegetative growth, the betaine content rising steadily from the root to the leaf. Furthermore, young, actively growing tissues (inner leaves) contain almost twice as much betaine as older, more mature tissues. These results of Cromwell *et al.* are in agreement with older data of Stanek (*51*) and other investigators. Although it is not entirely clear whether biologically betaine is formed in the roots and translocated to the leaves or made directly in the leaves,† the most

* *Note added in proof*: Recent studies provide welcome experimental evidence in support of this hypothesis (*45a, 45b*).

† Earlier work by Cromwell *et al.* (*52*) on the presence of choline oxidase in beet roots could not be confirmed and it was shown later that oxidase activity was due to the presence of contaminating bacteria. In recent experiments of Cromwell and Rennie (*53*) leaves of *Beta vulgaris* and *Atriplex patula* were infiltrated with

recent work by Cromwell and Rennie (*53*) carries the implication that betaine is formed in the leaf tissue from choline, by oxidation, and that exposure to light stimulates this conversion, perhaps through an increase in the intracellular oxygen content. At all events, betaine is utilized quite slowly by the plant, and thus continues to accumulate, reaching high concentration in older leaves. The accumulation of this material in relatively high concentration raises the question as to its function in the plant tissue. Betaine is known to participate in transmethylation reactions in vertebrates where it can be utilized for the biosynthesis of methionine (*54*, *55*). Whether this reaction occurs also in plants and more particularly in leaves is not known. Very little is known about the participation of betaine in other pathways of metabolism, but teleologically it might well be assumed that betaine fulfills some important function in the physiology of the plant.

In certain anaerobic bacteria, grown on betaine as a sole carbon source, betaine is transformed to dimethylglycine, while the methyl group which is formed in this reaction is utilized (possibly after oxidation) for the biosynthesis of acetate (*56*). Detailed formulation of this reaction would be of interest for it might disclose a new pathway for the utilization of the energy of the onium bond in betaine for biosynthetic purposes.

3. *Carnitine, Crotonobetaine, and γ-Butyrobetaine*

Although carnitine was discovered over 50 years ago (*57*, *58*) as a constituent of skeletal muscle the first clue as to its biological role came only in 1952 when it was recognized as identical with a vitamin, B_T (*59*), a growth factor essential for the meal worm *Tenebrio molitor* and certain other insects. A great deal of information is available on the natural distribution of carnitine, and the subject was competently reviewed very recently by Fraenkel and Friedman (*60*) whose paper should be consulted also for the chemistry of this compound and for a discussion of the biological effects of carnitine and its analogs. Carnitine is almost universally distributed, some exceptions being *E. coli*, the protozoan *Tetrahymena galeii*, and the hen's egg before development of the embryo. The tissues with the highest content are skeletal muscle and related tissues, such as the electric organ of the torpedo (*61*), which is a modified muscle end-plate.

In spite of the great advance represented by the recognition of the vitamin-like properties of carnitine, little is known about its biochemical

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solutions of choline or betaine aldehyde, the implication being that the choline oxidase system is present in the leaves.

role. Carnitine can undergo reversible biological acetylation (Eq. 9) by means of an enzyme isolated from pigeon liver (*62*):

$$\text{Carnitine} + \text{Acetyl-CoA} \leftrightarrows \textit{O}\text{-Acetyl carnitine} + \text{CoA} \qquad (9)$$

Since the reaction is readily reversible, if the enzyme catalyzing it were found also in muscle this pathway might indicate a function for carnitine as storehouse of energy-rich acetyl groups. Another and possibly significant lead on the biochemical function of carnitine is the finding of Fritz (*63*), that carnitine in concentrations as low as $6 \times 10^{-5}\,M$ increases the oxidation of palmitic acid by liver homogenates, and more specifically stimulates the formation of ketone bodies from higher fatty acids. The exact role played by carnitine in oxidation of higher fatty acids is not yet understood but it may be hoped that progress in this direction will be rapid as the field is an active one in biochemistry.

Nothing is known about the two betaines structurally related to carnitine, except that they are naturally occurring and, especially butyrobetaine, are found widely distributed both in invertebrates and in vertebrates (*64*). γ-Butyrobetaine is the only carnitine analog which competitively inhibits the utilization of carnitine by *Tenebrio molitor* (*60*).

4. *Ercinine and Ergothioneine*

Very little is known about ercinine but ergothioneine has received considerable interest among biochemists in recent years and its occurrence, distribution, biogenesis, and possible metabolic role will be discussed in some detail [see also Bell (*65*)].

a. *Origin and Distribution.* As implied by its name, ergothioneine was originally isolated and identified as a constituent of the ergot of rye by Tanret (*66*), and Barger and Ewins (*67*). In ergot it is found in concentrations as high as 250 mg./100 gm. No other source of this material was known until ergothioneine was discovered in pig blood (*68*) where its distribution is exclusively intracellular, being confined to the erythocytes. The content of ergothioneine in blood is very much smaller than in ergot and varies in different vertebrates between 1 and 5 mg./100 ml. (*69*). In the rat, at least, ergothioneine is not confined to red blood cells and several other tissues contain it in small amounts (*70*). Interest in this compound was revived in recent years as the result of the discovery by Mann and Leone (*71*) that this thiolbetaine is a normal constituent of boar semen into which it is secreted by the seminal vesicles. The concentration of ergothioneine in semen is particularly high, in some instances approaching the levels found in ergot.

Unlike blood, semen carries ergothioneine as an extracellular constituent in amounts between 30 and 250 mg./100 ml.

Although the efforts of earlier investigators (*72–74*) to demonstrate the presence of ergothioneine in a variety of plant materials yielded negative results, recently ergothioneine was found in oats (*75*) and it may be presumed that it is also present in other cereal grains. This discovery together with the earlier conclusion of the Cornell school that blood ergothioneine must be largely of dietary origin, since no evidence for the biosynthesis of ergothioneine by any animal species had been found (*76, 77*), clarifies considerably the question of the origin of ergothioneine in the blood of herbivorous vertebrates.

The problem has been clarified further by the finding that ergothioneine is a constituent of many species of microorganisms and that it is synthesized by several fungi (*78*). It is not yet clear whether the cereals in which ergothioneine has been found possess the ability to synthesize it or whether ergothioneine is derived from saprophytic fungi commonly present in cereals. The ergothioneine content of fungi grown on chemically defined media varies with the species, ranging between 4 and 85 mg. per 100 gm. of dried cells. Although many bacterial cells contain ergothioneine, no bacterial species is capable of synthesizing the compound (*78*). It has been shown that the presence of ergothioneine in the bacterial cell is due to the assimilation of this betaine from the growth medium. Some species, notably *Micrococcus pyogenes* accumulate it from the medium in unusually large amounts and concentrate it inside the cell to levels of 50 mg. per 100 gm. of dry cells.

b. *Biosynthesis.* The biochemical pathway and the precursors involved in the biosynthesis of this thiolbetaine has been elucidated in the main outlines by the work of Melville *et al.* (*79*) and of Heath *et al.* (*80–82*). It has thus become established that three amino acids, histidine, cysteine, and methionine, contribute to the different moieties of the ergothioneine molecule. Specifically, histidine supplies the imidazole nucleus and the side chain, while the sulfhydryl group is derived from cysteine and methionine contributes one or possibly all of the three methyl groups. The evidence suggests that the imidazole ring of histidine is not degraded in the conversion to ergothioneine and is incorporated intact. Thiolhistidine failed to serve effectively as a precursor of ergothioneine in *Neurospora crassa* (*79*), but this is not due to the inability of thiolhistidine to enter the cells. This would signify then that thiolhistidine is not directly on the synthetic pathway, and that methylation of histidine, either partial or complete, must precede the introduction of the sulfhydryl group. This is in agreement with the fact that thiolhistidine has never been shown to occur naturally,

whereas ercinine has been found in certain fungi (*83*, *84*). Cysteine is the most effective precursor of the sulfur atom of ergothioneine. When fed to *Neurospora*, cysteine competes very effectively with inorganic sulfate and in this regard is more effective than methionine, choline sulfate, or thioacetamide. Direct evidence of the utilization of cysteine sulfur for the biosynthesis of ergothioneine was supplied by the finding that when S^{35}-cysteine was used, the sulfhydryl group of ergothioneine was labeled with S^{35}. It has been shown that the methyl groups in ergothioneine are derived from methionine through a transmethylation reaction. This has not been demonstrated directly in a cell-free system but it may be inferred from the data of Melville *et al.* (*79*) who have shown in feeding experiments using methionine labeled with both C^{14} and deuterium in the methyl group that the methyl group is transferred as a unit. Using *Claviceps purpurea* Heath and Wildy (*80–82*) independently have obtained results which are in close agreement with this general pattern. It is thus well established that fungi are able to synthesize ergothioneine and it is quite possible that they are uniquely endowed in this respect.

It is of interest to point out that in cultures of *C. purpurea* grown on chemically defined media the formation of ergothioneine was confined to the conidia and it was found moreover that formation of sclerotia was not necessary for the production of the betaine. This fact, together with reported occurrence of ergothioneine in seeds and in semen suggested to Heath and Wildy the possibility that one of the biological functions of ergothioneine might be in some way related to germination. Although preliminary attempts to document this hypothesis by examining the effect of ergothioneine on the growth rate of cress seed were negative, this interesting idea seems to deserve further study.

In the course of their work, Heath and Wildy made an incidental observation which is of interest in connection with the over-all problem of alkaloid biosynthesis. Namely they observed that the characteristics of the culture *C. purpurea* changed during the course of the experiment with an increase in the ability to form ergothioneine taking place concomitantly with an increase in the pigmentation of the conidia.

Although the fact that ergothioneine is not synthesized in the mammal but is of dietary origin is now well established, it is becoming clear that there are other factors which govern the occurrence and distribution of ergothioneine in mammals. Thus, the distribution of ergothioneine in the accessory sexual secretions of the male does not fall into a clear pattern: its presence is not confined to boar semen as it has been found in the stallion, in the mole, and in the hedgehog but it is absent from the semen of man, bull, and ram. As for red blood cells, the Mac-

kenzie's (85) have found that the ability of the rat to accumulate ergothioneine in its erythrocytes is dependent both on the sex and age of the animal. In both sexes the ergothioneine level increases in the first 90 days of life thereafter remaining constant in the female, while doubling in the male. Moreover, this sex linked difference was not affected in the male by castration or by administration of estrogens, or both. On the other hand in female rats treated with testosterone the blood levels increased to approach the level found in the male. No explanation can be offered at the present time for this difference.

c. *Biochemical and Physiological Role.* The biochemical function of ergothioneine is still obscure. The presence of a thiol group *prima facie* suggests that this betaine may function as a reducing agent. As is well known there is a variety of naturally occurring sulfhydryl compounds and evidence is accumulating that they are not metabolically equivalent since some are specifically involved in well defined biochemical reactions. As yet, however, no specific role can be assigned to the thiol group of ergothioneine, but it may be recalled that as far back as 1911 Barger and Ewins (*67*) recognized the difference between the thiol groups of ergothioneine and cysteine. In the first place the former is relatively stable to alkali and is oxidized to sulfuric acid under conditions which result in the formation of a sulfonic acid from cysteine; it also differs from other thiol compounds in that it does not give a nitroprusside reaction or the Grote (*86*) reaction typical of thioureas and substituted thioureas (*87*). It will, however, form a disulfide under suitable conditions (*88*) although at physiological pH the equilibrium is completely towards the reduced state. As to its ability to form mixed disulfides there are conflicting reports (*88*, *89*) but at any rate the formation of mixed disulfides is considerably more difficult than with other thiols.

Ergothioneine is not a methyl donor and no reaction involving participation of its onium group has been discovered. Chemically, it will readily undergo a typical elimination reaction in alkali, yielding thiolurocanic acid and trimethylamine; recently it has been shown that an analogous enzymatic reaction takes place in bacteria (*90*).

The most suggestive clue to a biochemical role for this compound has recently emerged from the work of Grossman and Kaplan (*91*) on the base exchange reaction catalyzed by DPNase. These authors found that ergothioneine is required to initiate a base exchange reaction with *Neurospora* DPNase or erythrocyte RNase, which enzymes, in the absence of ergothioneine, will catalyze only the hydrolytic cleavage of DPN or NR respectively at the nicotinamide ribose linkage. (See also Section V,B,3). It may be particularly significant that, in *Neurospora*,

DPNase is found primarily in the conidia (*92*), which, as noted above for *C. purpurea,* are also rich in the ergothioneine-forming system.

III. Heterocyclic Onium Compounds

A. Saturated Heterocyclic Compounds

1. *Pyrrole Derivatives*

Stachydrine is found in a variety of plant materials, sometimes in high concentration as in orange leaves (13 μmoles per gram dry weight) (*93*) and generally accompanied by its higher homolog homostachydrine, the *N*-dimethylbetaine of pipecolic acid. The biogenesis of stachydrine has been investigated in alfalfa seedings (*94*) and a number of interesting features have emerged. It might be expected that proline would be the precursor of the pyrrole ring of stachydrine and some support for this hypothesis has been obtained. It has been found that administration of ornithine-C^{14} and pyridoxal resulted in labeling of both proline and stachydrine. However, the degree of C^{14} incorporation was small and clearly such results are indirect and far from compelling. More information is available as to the origin of the methyl groups. This was studied by measuring the incorporation of the methyl group of methionine into stachydrine. In young seedlings (15 days old) no incorporation of the methyl group of methionine was observed unless the nutrient medium was supplemented with folic acid (in addition to methyl-labeled methionine). Interpretation of these findings is difficult since no comparable data are available as to the rate and extent of incorporation of formate or other compounds which can serve as precursors of "one-carbon fragments." It is generally thought that folic acid coenzymes are not involved in transmethylation reaction, whereas it is well established that the *de novo* synthesis of a methyl group from one-carbon precursors requires the participation of tetrahydrofolic acid or of a compound derived from it (*95*). Therefore at the present time the best conclusion that may be drawn from the results of Wiehler and Marion (*94*) suggests that the biosynthesis of stachydrine, like the synthesis of choline, involves both *de novo* synthesis of a methyl group and transfer of the preformed methyl group of methionine. Although nothing is known about the biogenesis of betonicine, turicine, and homostachydrine, it may be presumed that it will follow the same pattern.

Upon heating, stachydrine undergoes a "Willstätter rearrangement" to the isomeric methylester of hygric acid. Hygric acid itself is found as a constituent in the nucleus of a great many alkaloids.

As to the biochemical or physiological significance of stachydrine or related compounds in this group nothing is known.

B. Heteroaromatic Compounds

1. *Thiazolium Compounds*

Thiamine is the most important member of this group and the one about which most is known. A discussion of its chemistry and biochemistry falls entirely outside the scope of this chapter but its cleavage by thiaminase will be discussed later as it has an important bearing on discussions of the energetics of the onium bond.

2. *Pyridinium Compounds*

In addition to DPN and TPN, which will not be discussed here, there are a number of naturally occurring alkyl-pyridinium compounds, such as *N'*-methylnicotinamide, and its oxidation product *N'*-methyl-3-carboxylamide-6-pyridone (*96*), *N'*-methylnicotinic acid, and *N'*-methylpicolinic acid.

Very little is known about the last compound: *N'*-methylpicolinic acid is found in high concentrations in mussels such as *Arca noae* (*97*) and *Mytilus californianus* Conrad, or the dinoflagellate *Gonyaulax catenella* on which it feeds (*98*), and in the nerves and other tissues of a variety of marine invertebrates (*99*). The distribution of the compound in the nerve muscles of these species is characteristic, and it has been suggested that by virtue of the ability of *N'*-methylpicolinic acid to form a mobile ion (H^+) when cleaved it might function in nerve conduction mechanisms (*100*).

N'-methylnicotinamide is formed from nicotinamide by a transmethylation reaction (*101, 102*) which represents the major pathway for the metabolism of nicotinamide in certain mammalian species. The details of the enzymatic aspects of the transfer reactions will be discussed in a later section. In man and rat, *N'*-methylnicotinamide is metabolized further to *N'*-methyl-3-carboxamide-6-pyridone and present evidence suggests that this pathway accounts for 80% or more of the catabolism of *N'*-methylnicotinamide (*103*). In other mammals, however, nicotinamide is excreted directly or converted to a number of different metabolites.

N'-methylnicotinamide is rapidly excreted in the urine owing to a renal excretory mechanism involving tubular secretion (*104*).

The methylation of nicotinamide does not seem to be confined to animal species as it occurs also in corn where this metabolic characteristic is under genetic control. In man *N'*-methylnicotinamide and the

corresponding pyridone are also important products in the metabolism of the amino acid tryptophan, but this is not the case in other mammals such as dogs, cats, and rats (*105*).

IV. Sulfonium Compounds

From a chemical point of view sulfonium compounds as a group may be considered analogous to the quaternary nitrogen compounds. It is interesting to observe that although sulfonium compounds had been known to chemists for almost 100 years, and quaternary ammonium compounds have been recognized in nature for the same length of time, sulfonium compounds have not been known to occur biologically or to play a part in intermediary metabolism until quite recently. At the present time only 3 sulfonium compounds have been found to occur naturally: dimethylpropiothetin, S-adenosylmethionine, and S-methylmethionine. It is of interest that the suggestion that sulfonium compounds might play a part in biological processes (*106*) anteceded the isolation of such compounds from naturally occurring materials.

A. Natural Occurrence and Chemistry

1. *Dimethylpropiothetin*

The first* demonstration of the natural occurrence of sulfonium compounds was the outcome of a search for the precursor of the dimethylsulfide which is evolved from certain marine algae.

Challenger and Simpson (*109*) isolated dimethylpropiothetin

$$(CH_3)_2\overset{\oplus}{S}—CH_2—CH_2—COO^-$$

from *Polysiphonia lanosa*, and conclusively proved that this sulfonium compound was the precursor of the evolved dimethylsulfide. Formation of dimethylsulfide and acrylic acid on alkaline treatment of dimethylpropiothetin had been reported earlier by Holmberg (*110*) and alkaline fission of sulfonium compounds is generally well known. Since liberation of dimethylsulfide from *P. lanosa* is associated with exposure of the algae to environmental changes (air, distilled water, or detachment from its host, *Ascophyllum nodosum*) but is not noticed after immer-

* Neuberg and Grosser (*107*) in 1905 tentatively identified as methyldiethylsulfonium hydroxide a compound from dog urine capable of evolving diethylsulfide on mild warming with alkali. However, authentic methyldiethylsulfonium hydroxide does not decompose to diethylsulfide with warming in NaOH and even at 100°, it cleaves primarily to methylethylsulfide (*108*). Thus, the exact identity of the compound described by Neuberg and Grosser remains to be established.

sion in boiling water, Challenger and Simpson suggested that the evolution of dimethylsulfide is an enzymatic process.

This suggestion was recently confirmed by Cantoni and Anderson (*111*) who established that cell-free extracts of *P. lanosa* catalyze the cleavage of dimethylpropiothetin according to Reaction 10.

$$(CH_3)_2\overset{\oplus}{S}-CH_2-CH_2-COO^- \rightarrow CH_3-S-CH_3 + CH_2=CH-COO^- + H^+ \quad (10)$$

Thus, it is noteworthy that the products of the enzymatic cleavage are the same as those formed by alkaline fission.

Dimethylpropiothetin is present in a variety of algae and its distribution seems to be related to the natural habitat of the algae in which it is found (*112*) in that it appears to be confined mainly to algae living in tidal waters and to be absent in fresh-water algae or in algae living in the high seas.

The fact that a very mobile ion, namely H^+, is formed as a result of the enzymatic cleavage of dimethylpropiothetin together with the characteristic distribution pattern and the presence of dimethylpropiothetin in very high concentration in certain algal species [*P. lanosa* may contain as much as 40–60 μmoles per gram wet weight (*113*)] suggested to Cantoni (*100*) that the biological function of this sulfonium compound might be related to the maintenance of the osmotic equilibrium of the algae.

Although the enzymatic cleavage of dimethylpropiothetin has been studied only in *P. lanosa* it probably occurs in other algae and in other species as well, as Y. Cheng Lin (*114*) has found dimethylsulfide evolution from dimethylpropiothetin in cultures of *Polysiphonia notatum* and *Scopulariopsis brevicaulis*.

A compound with the same elementary composition as dimethylpropiothetin, namely the methylester of thiomethylpropionic acid

$$CH_3-S-CH_2-CH_2-CO-O-CH_3$$

has been isolated from the juice of the Hawaiian pineapple by Haagen-Smit *et al* (*115*). It is interesting to point out that biologically, as well as chemically, thiomethylpropionic acid methylester could arise from dimethylpropiothetin through a "Willstätter migration," (see above, Section I,B).

The discovery of the natural occurrence of dimethylpropiothetin coincided with the recognition that the thetins are compounds of potential biological importance in transmethylation reactions in vertebrates. This was first established by Welch (*116*) and later investigated more extensively by Maw and du Vigneaud (*117*) who found that dimethylpropiothetin, or its lower analog dimethylacetothetin, when

added to the diet could serve as sources of "labile" methyl groups for the rat and other vertebrates.* The ability to serve as dietary methyl donors correlates well with the ability of these compounds to act as sources of "mobile" methyl group in the enzymatic synthesis of methionine (*119, 120*). Whether dimethylpropiothetin has a similar function in the species in which it is found is not known. Nothing is known about the biological origin or mechanism of formation of dimethylpropiothetin, but it might well arise by a reaction between acrylyl-CoA and dimethylsulfide by analogy with the reaction between acrylyl-CoA and ammonia discussed above (*30*). The chemical and biochemical requirements for "methyl" group mobility and its relationship to "onium" configuration will be discussed in a later section.

2. *S-Adenosylmethionine*

S-Adenosylmethionine was discovered and identified by Cantoni (*121*) as the product of the enzymatic activation of methionine for transmethylation. It is formed enzymatically from methionine and adenosine triphosphate in liver and other tissues (*122*), in yeast (*123, 124*), *Escherichia coli* (*125*), and probably a variety of other microorganisms, as well as in *Chlorella* (*126, 127*) and perhaps other green algae.†

a. *Chemistry.* The structure of S-adenosylmethionine, first proposed on the basis of biochemical evidence (*121*) and later confirmed both by degradation (*129*) and by synthesis (*130*), is shown in Fig. 6.

* The term "labile methyl compound" as defined by du Vigneaud is a *nutritional* concept, in that it defines the ability of the compound to furnish "methyl groups" for various synthetic reactions, when added to the diet. The increasing complexity of our knowledge of transmethylation reactions and a fuller understanding of their mechanism requires some amplification of the terminology. Thus it has been suggested (*118*) that a methyl, or alkyl, group directly attached to a neutral onium pole be defined as "mobile methyl group" to indicate its high reactivity in enzymatic group-transfer reactions.

† The methionine-activating enzyme of liver and yeast will also utilize ethionine, and the selenium analog of methionine (*128*). The activation of ethionine proceeds at a slower rate and the product is S-adenosylethionine, a compound with chemical characteristics very similar to S-adenosylmethionine. Until it can be shown that ethionine is a naturally occurring amino acid, S-adenosylethionine will have to be considered as an analog of adenosylmethionine with potentially interesting features as an antimetabolite. Selenomethionine, on the other hand, is utilized as well or better than methionine (*128*). The product *Se*-adenosylselenomethionine can serve as a methyl donor biologically and satisfy the requirements for organic selenium in the rat (*128a*). Since selenomethionine is a naturally occurring methionine analog it is probable that *Se*-adenosylselenomethionine is likewise a natural product.

The presence of the sulfonium configuration, as is well known, confers on a compound an additional center of optical activity, and stereoisomers of sulfonium compounds have been separated by classical chemical procedures (*131*, *132*). Synthetic S-adenosylmethionine, whether prepared by chemical methylation of S-adenosylhomocysteine (*133*) or by condensation of 5′-methylthioadenosine with 2-amino-4-bromobutyric acid would be expected to be a mixture of diastereoisomerides, while it could be expected that the natural compound would be optically homogeneous. Recently both sulfonium diastereoisomers of S-adenosyl-L-methionine have been prepared by different enzymatic procedures, based on the fact that the synthetic compound differs from the natural compounds in that it is utilized only to 50% in the enzymatic methylation of guanidinoacetic acid (whereas under similar conditions natural S-adenosylmethionine is utilized to 90–100%). These findings suggested that one of the two stereoisomers of S-adenosyl-L-methionine

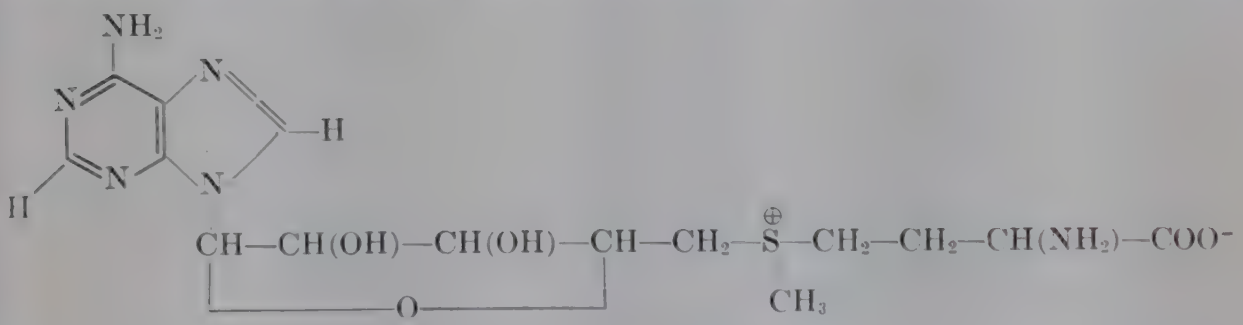


FIG. 6. S-Adenosylmethionine.

might be inactive as a methyl donor in this and possibly other enzymatic systems. The optical rotation of the two sulfonium diastereoisomers has been determined and compared to that of the synthetic compound (*134*). It was found that the optical rotation of samples of S-adenosyl-L-methionine prepared by the chemical methylation of adenosylhomocysteine was $\{[\alpha]_{5890}^{24^\circ}(+)52^\circ\}$; this value is significantly different from that of S-adenosylmethionine formed by the methionine activating enzymes of liver and yeast which gave identical values of $\{[\alpha]_{5890}^{24}$ approx. $(+)48^\circ\}$. Finally the optical rotation of the epimeric sulfonium isomer of S-adenosyl-L-methionine was $\{[\alpha]_{5890}^{24^\circ}(+)57^\circ\}$. This isomer,* which may be designated as

* In considering the four diastereoisomerides possible in S-adenosylmethionine, it will be convenient to use the conventional designations D- and L- to describe the configuration about the 2-carbon atom, while using the symbols (+) and (−) to indicate the contribution of the sulfonium group to the rotation of the whole molecule. The completely racemic compound will be described as (±) S-adenosyl-DL-methionine and the natural, or enzymatically formed compound as (−)S-adenosyl-L-methionine.

(+)S-adenosyl-L-methionine was obtained, as indicated above, by allowing (+)S-adenosyl-L-methionine to react as completely as possible (or 50%) with guanidinoacetic acid in the presence of guanidinoacetic acid methylpherase and reisolating the inactive sulfonium compound, which was shown to be otherwise identical to enzymatically formed adenosylmethionine. (+)S-adenosyl-L-methionine is inactive as a substrate in a number of reactions utilizing S-adenosylmethionine (*134*).

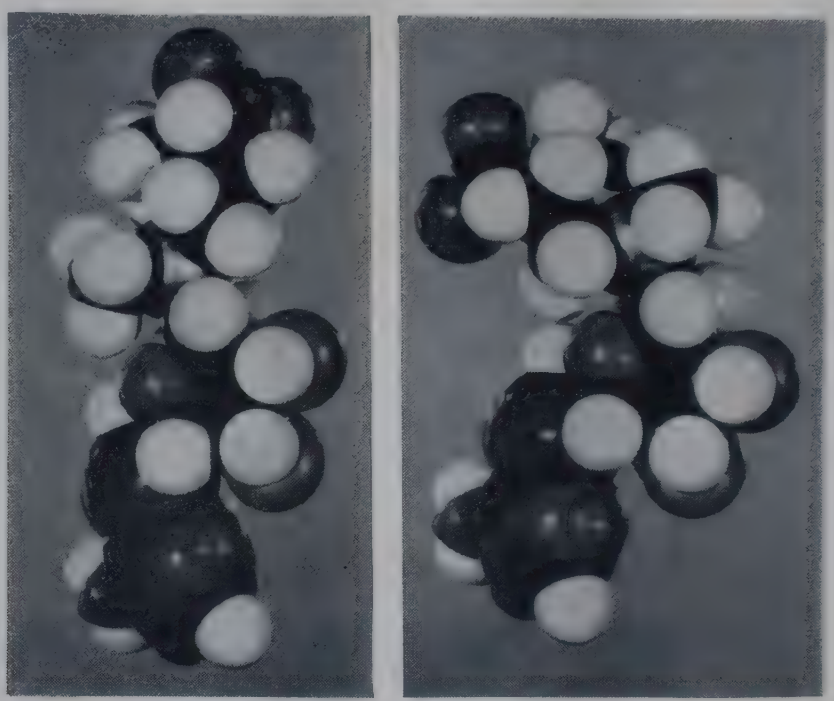


FIG. 7. Molecular models of S-adenosylmethionine. The 2 models differ in the orientation of the substituents around the sulfonium center.

Figure 7 shows molecular models of the two sulfonium diastereoisomers of S-adenosyl-L-methionine. There are, of course, other centers of optical activity in adenosylmethionine such as the α-amino group, the glycosidic bond, etc. but the two models shown here differ only at the sulfonium center.

A careful study of some of the chemical properties of S-adenosylmethionine (*135*) revealed some novel and rather unique features. The compound is rather stable in strong acids at low temperature; at higher temperatures the stability of its glycosidic bond is very nearly identical with those in adenosine and methylthioadenosine. On the other hand in alkali it is exceedingly labile and is hydrolyzed very rapidly (10 minutes in 0.1 *N* NaOH at 25°) with the formation of adenine and S-ribosylmethionine. At pH between 7 and 10 the ultraviolet absorption spectra of adenine and S-adenosylmethionine differ significantly, these differences being greatest at 250 mμ where the molecular extinction coefficient of adenine is approximately 55% smaller than that of the sul-

fonium nucleoside; spectrophotometric analysis of this alkali-catalyzed hydrolytic reaction and a study of its kinetics thus becomes possible. The effect of incubation in 0.1 *N* NaOH at 25° on the ultraviolet absorbency of S-adenosylmethionine at 250 mμ is shown in Fig. 8 drawn from data of Schlenk. It must be pointed out that one of the products of the alkaline cleavage, S-ribosylmethionine is itself a sulfonium compound and as such is labile to alkali [methionine and another unidentified product (or products) are formed as a result of its hydrolysis at pH 11].

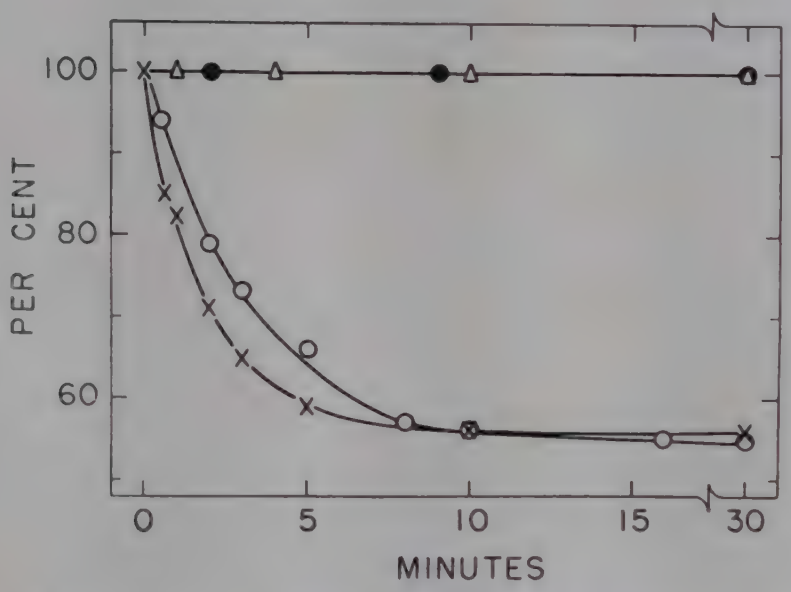


FIG. 8. The effect of 0.1 *N* NaOH at 25° on S-adenosylmethionine (×); dimethyladenosylthetin (○); adenosine (●); and 5′-methylthioadenosine (Δ). The optical density at 250 mμ was recorded; the decrease to 55% of the original density value indicates complete hydrolysis of the glycosidic bond. [From Parks and Schlenk (*135*)].

The extreme lability of the glycosidic bond in adenosylmethionine to alkali has few analogies in the chemistry of purine nucleosides (or nucleotides) and is a finding of considerable interest. Adenosine, methylthioadenosine, or adenosylhomocysteine are not hydrolyzed appreciably under these conditions. As can be seen by studying the molecular model of adenosylmethionine the sulfonium center is in close proximity to the glycosidic bond. The inductive effect of sulfonium groups on neighboring sites is well known. Here, however, the bond which is labilized is 5 carbon atoms removed and herein, and in the fact that the sulfonium configuration is not lost in the reaction, lies the novel characteristic of this cleavage, which is exhibited also by another, synthetic, adenosylsulfonium compound, namely S-adenosyldimethylthetin (*135*) (Fig. 9). It appears that this chemical lability finds a counterpart in biological systems as indicated in the recent finding of

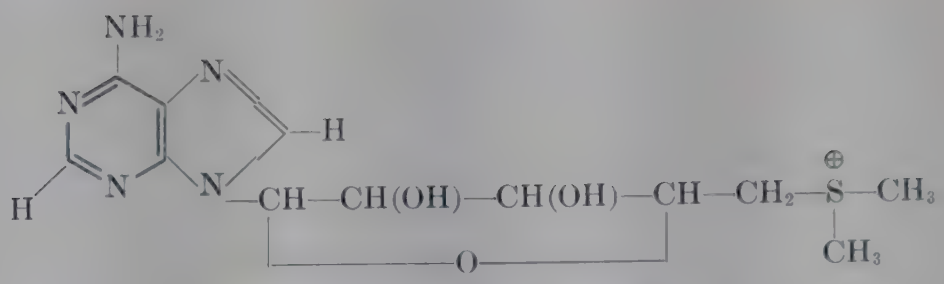
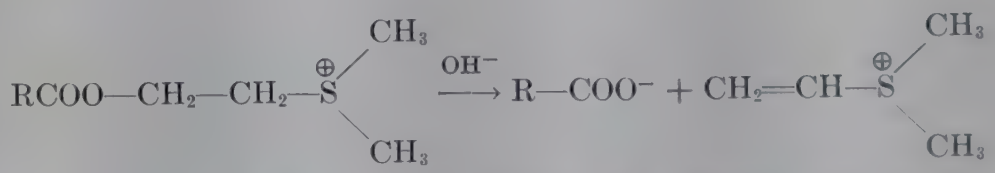


FIG. 9. S-Adenosyldimethylthetin.

Schlenk (*136*) that in certain bacterial species adenine C^{14} exchanges with the adenine moiety of adenosylmethionine as outlined in Eq. 11.

$$\text{Adenine-}C^{14} + \textit{S}\text{-Adenosylmethionine} \rightleftarrows \text{Adenine} + \textit{S}\text{-Adenosyl-}C^{14}\text{-methionine} \quad (11)$$

One chemical analogy of the cleavage under discussion is the alkaline fission of 2-aroyloxethyldimethylsulfonium compounds (*137*) according to Eq. 12.



(12)

Another analogy is seen in the report of Lawley that on mild hydrolysis (pH 7 and 37°) 7-methyldeoxyguanylic acid yields 7-methylguanine and deoxyribose-5-phosphate. The mechanism of the reaction seems to involve the initial formation of 7-methyldeoxyguanosinium-5′-phosphate (Fig. 10, I) or its isomer with the charge on N-9 (Fig. 10, II), followed by elimination of the side chain (*138*).

At pH 4–7 and 100°, S-adenosylmethionine cleaves hydrolytically and almost quantitatively with the formation of methylthioadenosine and 2-amino-4-butyrolactone which under these conditions rapidly hydrates to homoserine (*129*).

b. *Natural Distribution.* Although S-adenosylmethionine was first discovered as the product of a reaction catalyzed by a liver enzyme, this compound does not accumulate normally in the liver or other mammalian tissues to a measurable extent. On the other hand it has been known for almost 50 years that yeast contains a sulfur nucleoside, whose structure has been shown to be identical with methylthioadenosine (Fig. 11).* In fact the elucidation of the structure of S-adenosylmethionine has been greatly aided and stimulated by the previous knowledge on the natural occurrence of methylthioadenosine. More recently the important work of Schlenk and his group emphasized the fact that the thiomethyl group of methylthioadenosine originates from the thiomethyl group in methionine (*139*; see also *140*). Since under

* See references *138a* and *b*.

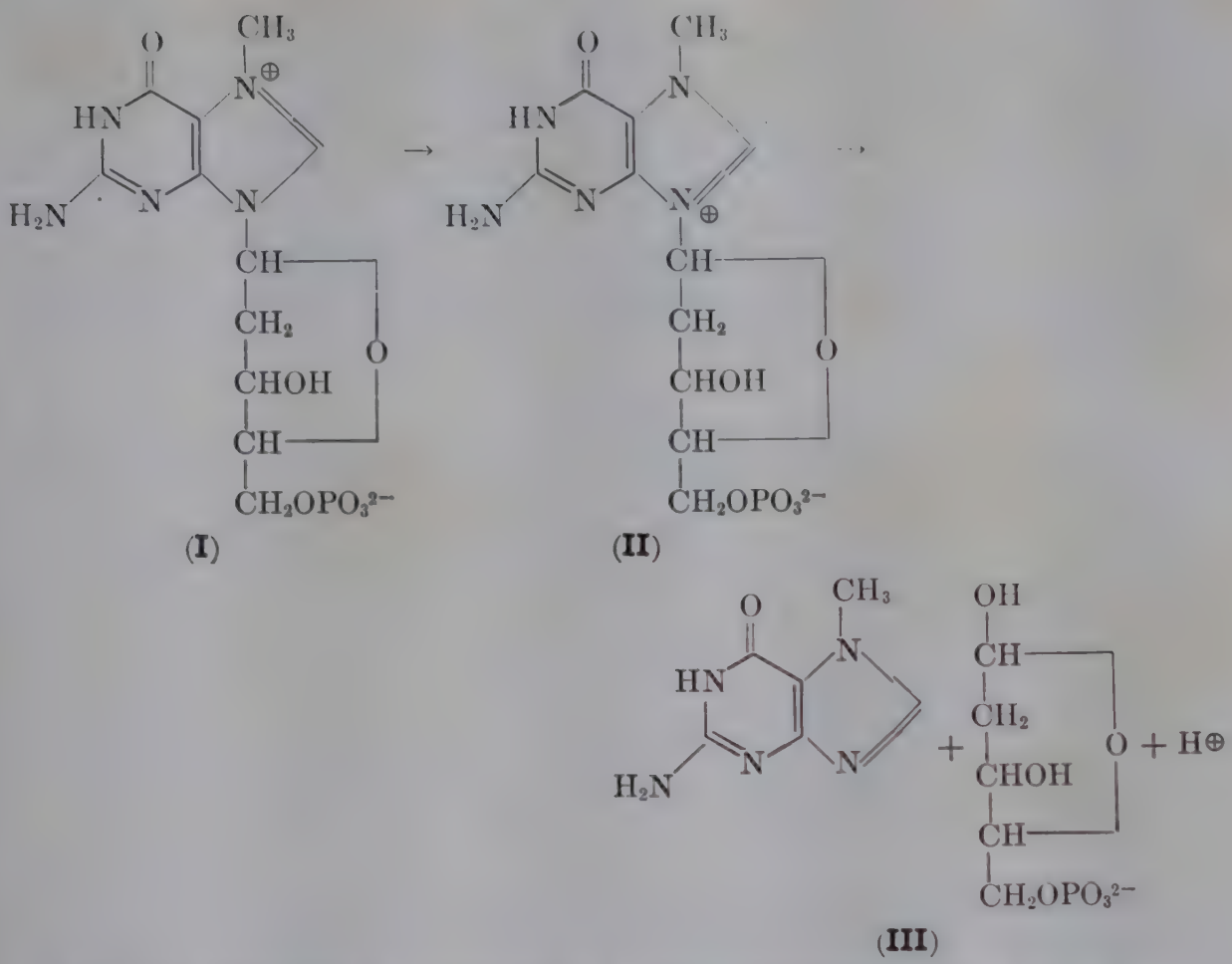


FIG. 10. Mechanism of hydrolysis of 7-methyldeoxyguanylic acid at pH 7.0.

conditions originally employed for the preparation of methylthioadenosine from yeast, S-adenosylmethionine would break down to methylthioadenosine and homoserine, it was suggested by Baddiley *et al.* (*129*) that the yeast nucleoside was in reality adenosylmethionine from which the simpler sulfur-containing nucleoside would arise during the

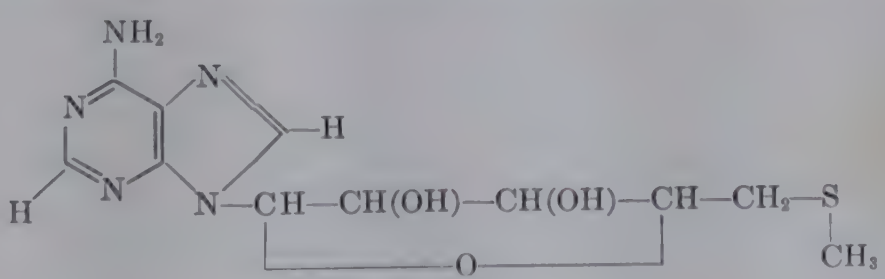


FIG. 11. Methylthioadenosine.

extraction procedure. Recent work of Schlenk and de Palma (*141*), and of Mudd (*142*) in the author's laboratory has confirmed this hypothesis in that it has been shown that, if care is taken to exclude the possibility of hydrolysis of the sulfonium compound during extraction, yeast grown in the presence of methionine contains large amounts of S-adenosylmethionine and only little methylthioadenosine (Table IV).

This latter compound, however, is not exclusively a chemical artifact for it can also arise biologically in yeast and other microorganisms through the action of at least two types of enzyme systems: the propylaminepherase of Tabor *et al.* (*143*) which, after the decarboxylation of adenosylmethionine catalyzed by a separate enzyme, transfers the remaining propylamine moiety to a receptor polyamine; and the adenosylmethionine-cleaving enzyme studied by Mudd (*144*) and by Shapiro and Mather (*145*).

TABLE IV

EFFECT OF METHOD OF EXTRACTION ON THE SULFUR NUCLEOSIDES OF YEAST (*T. utilis*)[a]

Methods of extraction	Methylthioadenosine[b]	*S*-Adenosylmethionine[b]
Water at 98° for 5 minutes	15	4.6
Water at 70° for 5 minutes	4.5	7.2
Treatment with liquid nitrogen, followed by extraction with H_2O	1.6	15.3

[a] From data by F. Schlenk and P. G. de Palma, *J. Biol. Chem.* **229,** 1037 (1957).
[b] Micromoles per gram.

A considerable amount of information is available as to the effect of nutritional and metabolic conditions on the concentration of adenosylmethionine and/or methylthioadenosine in yeast, and in reviewing these data the assumption will be made that the concentration of the latter is in reality an expression of concentration of the sulfonium compound unless both compounds have been determined separately. When *Torulopsis utilis* is grown in the absence of methionine, but in the presence of sulfate in quantities adequate to support protein synthesis at optimal rate, the formation of adenosylmethionine and its concentration in yeast is low (1 μmole/gm.). Addition of various sulfur-containing supplements results in a 20-fold increase in the concentration of adenosylmethionine in *T. utilis* as shown in Table V which is derived from a recent paper of Schlenk and de Palma (*141*). It is noteworthy that L-methionine is not as good a precursor as D-methionine; L-homocysteine and S-methyl-L-methionine have little effect by themselves but their combination proved the most effective way of supplying the precursor required for adenosylmethionine synthesis in yeast. Under these circumstances the compound accumulates in truly remarkable amounts: thus it may be calculated that the value of 20 μmoles/gm. is equivalent to 3% of the dry weight of yeast. The reasons for the superiority of the S-methyl-L-methionine + L-homocysteine combination over the equimolar amounts of methionine may be related to the ability of the sulfonium compound to act as a methyl donor for homocysteine and thus

maintain a constant and high intracellular methionine concentration. Since S-adenosylmethionine accumulation requires the utilization of an equimolar amount of ATP, the accumulation of large amounts of sulfur-containing nucleosides in yeast fed a suitable sulfur supplement may be considered as an intracellular trapping mechanism for adenine groups even though at low concentrations of methionine it is likely that adenine nucleosides and/or nucleotides are utilized preferentially for nucleic acid synthesis rather than for S-adenosylmethionine forma-

TABLE V

EFFECT OF SULFUR SUPPLEMENTS ON *S*-ADENOSYLMETHIONINE CONCENTRATION IN YEAST (*T. utilis*)[a]

Sulfur supplement	Concentration (micromoles/milliliter)	Yield of cells (grams/100 ml.)	*S*-Adenosylmethionine concentration in yeast (micromoles/gram)
None	—	4.0	1.1
L-Methionine	2	4.0	2.5
	4	3.9	4.0
	10	2.7	5.8
D-Methionine	2	3.9	3.4
	4	3.9	5.2
	10	3.7	8.5
S-Methyl-L-methionine	4	3.7	2.1
L-Homocysteine	4	4.0	3.5
S-Methyl-L-methionine +	2 + 2	3.4	12.4
L-homocysteine	4 + 4	3.0	20.1

[a] From data by F. Schlenk and P. G. de Palma, *J. Biol. Chem.* **229,** 1037 (1957).

tion. As to the source of the adenine, it must be obtained largely through *de novo* synthesis since Schlenk found that neither adenine nor adenosine had any effect on S-adenosylmethionine formation by intact yeast, and Schmidt *et al.* (*146*) reported that methionine feeding resulted in large increases not only of the acid soluble adenine but also of "total" RNA adenine. Moreover the striking stimulation of adenine formation observed in the presence of methionine is accompanied by a very large stimulation on the biosynthesis of guanine and pyrimidine nucleosides (Fig. 12).

Ethionine is almost as effective as methionine in promoting the accumulation of sulfur nucleosides [S-adenosylethionine and its breakdown product ethylthioadenosine (*147*)] but it differs strikingly from methionine in its effect on the other bases. With ethionine the newly formed adenine was almost quantitatively accounted for by the increase on the acid-soluble nucleosides, whereas the formation of guanine and other pyrimidines was either not significantly influenced or con-

siderably depressed (Fig. 12). In this case then, it must be assumed that the formation of the sulfur nucleoside takes precedence over nucleic acid formation and possibly this trapping mechanism accounts for some of the toxic effects of ethionine in yeast.*

As already indicated above, another sulfonium compound related to adenosylmethionine has recently been discovered by the important work of Tabor *et al.* (*143*), namely S-adenosylthiomethylpropylamine,

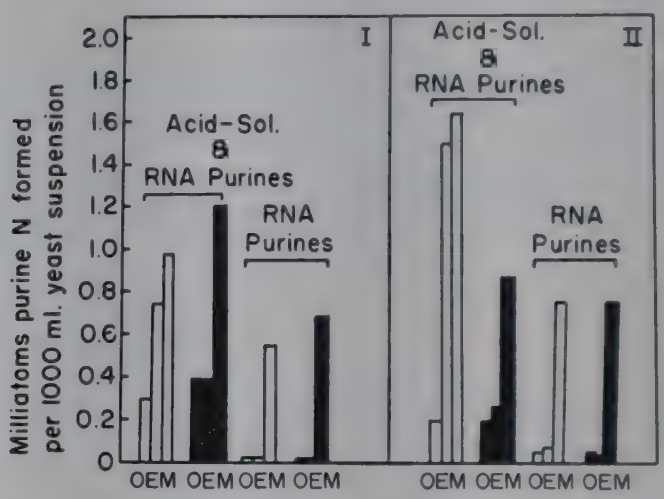


FIG. 12. The effect of methionine and ethionine on the formation of ethionine and guanine groups in bakers' yeast. The bars represent the increment of purine groups over initial level. White bars–adenine groups and black bars–guanine groups. *Abbreviations:* O—no addition; E—ethionine; M—methionine. Concentration of amino acid, Expt. 1: 0.13 m*M*; Expt. 2: 6.5 m*M*. [From data by Schmidt *et al.* (149)].

which is formed from S-adenosylmethionine by enzymatic decarboxylation. Its role as an intermediate in spermidine synthesis has been demonstrated conclusively, and its isolation may be expected in the near future.

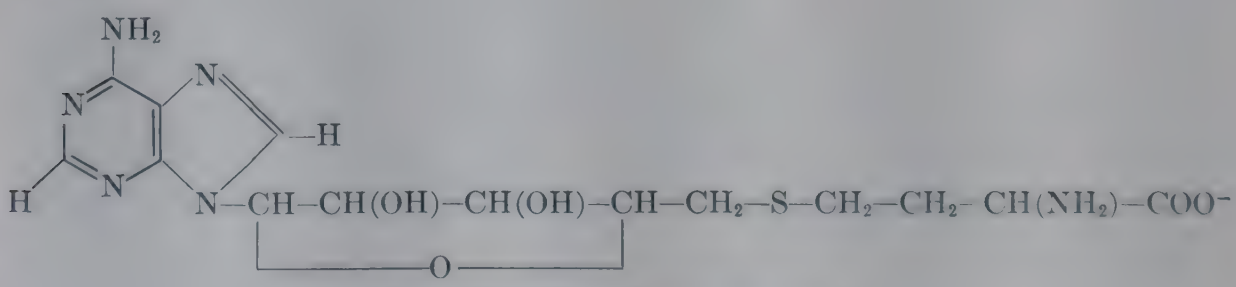


FIG. 13. Adenosylhomocysteine.

* It may be mentioned in passing that in addition to S-adenosylmethionine, S-adenosylethionine, and methylthioadenosine, another sulfur-containing nucleoside has been described (*148*) and synthesized (*149*), namely adenosylhomocysteine shown in Fig. 13. This compound is the product of the enzymatic demethylation of adenosylmethionine and is also formed enzymatically by a second pathway, namely the condensation of adenosine and homocysteine (*150*).

3. S-*Methylmethionine*

S-Methylmethionine, the third naturally occurring sulfonium compound, has so far been found only in plants. It was first isolated by McRorie *et al.* (*151*) who identified this analog of methionine with a natural material found in cabbage juice which is capable of counteracting the toxicity of sulfanilamide in *E. coli* (*152*). It is interesting to point out that under conditions of limiting methionine biosynthesis S-methylmethionine is 2–7 times more active than molar equivalents of methionine in reversing sulfanilamide inhibition. This observation suggests that S-methylmethionine may serve not only as a precursor of methionine but also as a methyl donor or as a precursor of some essential cell constituent, possibly adenosylmethionine as was just noted for yeast. In cabbage S-methylmethionine is found in concentrations similar to those quoted for S-adenosylmethionine in yeast (2 μmoles per gram dry weight). S-methylmethionine is found in a variety of other plants, such as (named in the relative order of concentrations) parsley, turnip greens, turnip, pepper, carrot, onions, lettuce (*151*), and asparagus (*153*).

As would be expected the chemical properties of S-methylmethionine resemble those of adenosylmethionine and of dimethylpropiothetin. It is quite stable to acid; at pH 7 or higher it decomposes rapidly to dimethylsulfide and 2-amino-4-butyrolactone, which then hydrates to homoserine.

Little is known about the biological origin and function of S-methylmethionine. As indicated, it is able to replace methionine both in microorganisms and in vertebrates (*154*), and in fact, under some conditions it is superior to methionine as a growth factor for methionine auxothrophs of *E. coli*, *L. arabinosus*, and *L. casei*, *Proteus*, and *Aerobacter aerogenes* (*155, 156*).*

These results, like the ones of McRorie (*152*) and those of Schlenk reviewed above (*141*), indicate that in certain cases S-methylmethionine is a more direct precursor of an active intermediate or an essential cell constituent than is methionine itself, but give no clue as to the nature of the compound involved. It is possible to visualize at least two alternative mechanisms to explain these observations. In the first place it is now known that S-methylmethionine can transfer enzymatically one of its methyl groups to homocysteine (*157*), and in this way dietary supplementation with S-methylmethionine may result in a higher intra-

* However, *Streptococcus faecalis R*, *Leuconostoc mesenteroides* P-60, some strains of *E. coli* and of *Neurospora crassa* do not have the ability to utilize S-methylmethionine *in lieu* of methionine.

cellular steady state concentration of methionine. Alternatively it might be postulated that S-methylmethionine can react enzymatically with S-adenosylhomocysteine to form adenosylmethionine directly, although there is at present no positive evidence that such a reaction takes place.

As has been noted, the chemical degradation of both adenosylmethionine and S-methylmethionine can result in the formation of

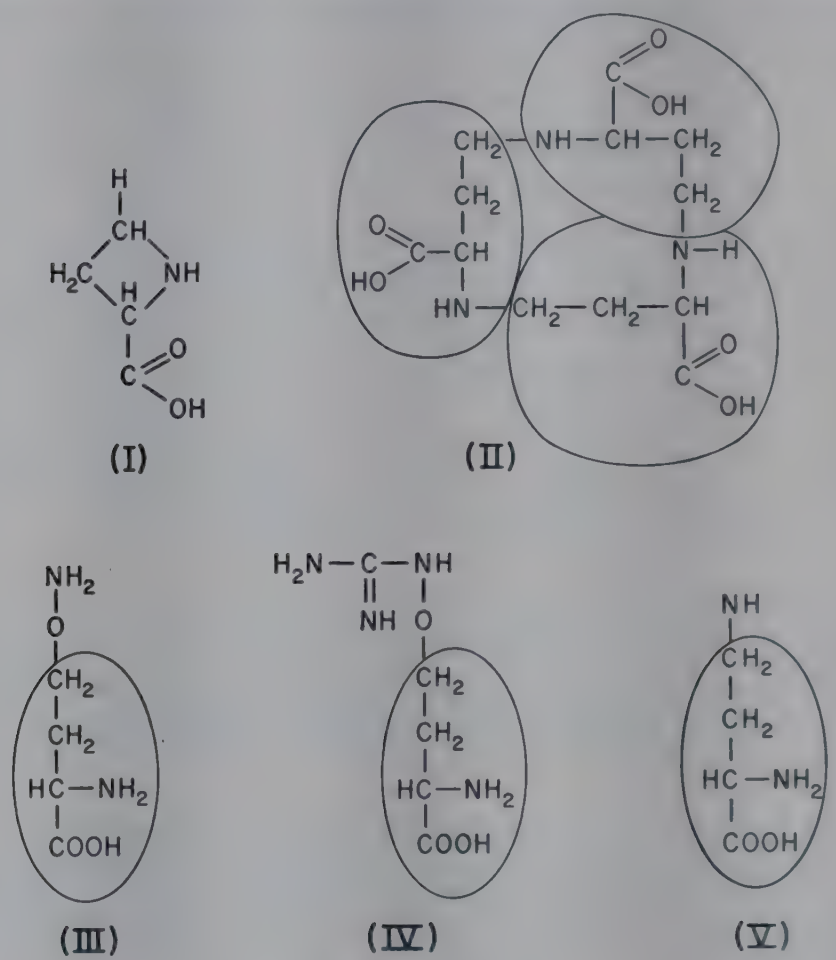


FIG. 14.

2-amino-4-butyrolactone. Moreover in the case of adenosylmethionine there is evidence that an analogous reaction takes place enzymatically. It is therefore of interest to point out that homoserine is found in large amounts in certain plant materials such as the Jack bean, the pea, etc. (*158*). Moreover, Fowden (*159*) has identified in extracts of plants belonging to the families Liliaceae, Agavaceae, and Amaryllidaceae a cyclic imino acid, azetidine-2-carboxylic acid (Fig. 14,I), which may be considered as a cyclic derivative of homoserine. It is noteworthy that in plants of the Liliaceae family azetidine-2-carboxylic acid is the predominant amino acid, being present in an amount equivalent to about 70% of the total free amino and imino acids of the extracts. On the basis of more recent work, azetidine-2-carboxylic acid appears to be identical with the compound found by Virtanen and Linko in *Polygonatum*,

originally thought to be a more complex cyclic structure with a molecular weight of 303 (Fig. 14,II) (*160*). Finally canaline, canavanine, and 2,4-diaminobutyric acid (shown in Fig. 14, III, IV, and V) contain a 4-carbon chain which may be derived directly from methionine. Both canaline and canavanine are found in large amounts in the Jack bean (*161*), and certain other plant materials (*162*), and 2,4-diaminobutyric acid, known earlier as a constituent of the polymyxins antibiotic (*163*), has been recently identified in rhizomes (*164*). It is thus tempting to suggest that in all these cases the 4-carbon chain arises through an enzymatic transalkylation reaction from one of the two naturally occurring sulfonium derivatives of methionine: S-methylmethionine or S-adenosylmethionine.

V. Metabolism and Enzymology of Onium Compounds

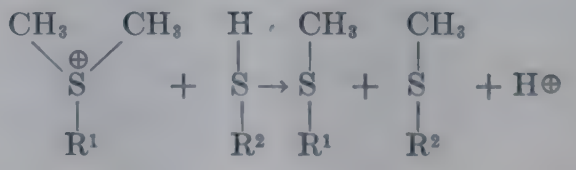
As noted in a preceding section the biological activity of sulfonium compounds was discovered in the course of investigations on the relationship between chemical structure and the biological ability to replace methionine for the biosynthesis of choline and other methylated compounds. It was found in the course of these investigations that betaine, dimethylacetothetin, dimethylpropiothetin, and S-methylmethionine can replace methionine as sources of methyl donors in the adult rat. It will be immediately obvious that there is a clear-cut chemical difference between methionine and the compounds in this group in that these are all onium compounds whereas methionine itself is a thioether. This chemical difference is reflected in the different biochemical behavior of these two types of methyl donor compounds. Whereas an onium compound such as dimethylthetin can transfer one of its methyl group to an acceptor compound without requiring a source of energy, the transfer of the methyl group of methionine requires the participation of ATP (*55*). This observation in turn led to the discovery that in reality activation of methionine by ATP is a prerequisite to methyl transfer and that as a result of this activation methionine is transformed into a sulfonium compound which is characterized by the ability to function as a methyl donor directly, that is, in the absence of an energy source. Before discussing in more detail the enzymology of onium compounds, the energetics of the onium bond will be reviewed.

A. Energetics of the Onium Bond
(Written in collaboration with Dr. J. Durell)

The concept has been independently stated in recent years by several investigators (*23*, *162*) that compounds with onium groups, par-

ticularly quaternary ammonium and sulfonium compounds, serve biologically as a source of utilizable chemical energy analogous to compounds with pyrophosphate and acyl phosphate linkages. The concept evolved independently from two different lines of research: (*a*) the study of base exchange reactions, particularly the thiaminase reaction (*165*); and (*b*) the study of transmethylation reactions (*23*). In the first case, it was observed that a number of nitrogenous bases could exchange for the thiazolium moiety of thiamine, thus indicating that this onium compound served as the source of energy for synthetic reactions. Moreover, it appeared that the reaction was irreversible unless the new linkage formed also resulted in the formation of an onium group. In the area of transmethylation, with the discovery of *S*-adenosylmethionine (*121*), it became apparent that compounds acting as donors of mobile methyl groups were invariably methyl-onium compounds, again pointing to the utilization of the energy of the onium group for chemical synthesis. The concept that the driving energy for these reactions resided in the reduction of the onium pole was elaborated by Woolley (*162*). Both Cantoni (*23*) and Kalckar (*166*) considered it more important to stress the significance of the liberation of a proton in a buffered solution. The two explanations differ only formally, because the liberation of a proton in aqueous solution results in the formation of a hydronium ion, and the reaction of this ion with buffer can, if one wishes, be viewed as the reduction of an onium group and the oxidation of the buffer.

Any discussion of the energetics of transmethylation is necessarily handicapped by the unfortunate fact that no equilibrium measurements have been obtained on any transmethylation reaction. It has been possible, however, to determine calorimetrically the enthalpy change associated with two transmethylation reactions catalyzed by purified thetin-homocysteine methylpherase (*119*). With certain reasonable assumptions as to the entropy changes involved, the free energy changes of these reactions have been estimated. The first of these is Reaction 13.*



(13)

* Throughout this section, the symbols R^1 and R^2 have the following meanings:

$R^1 = -CH_2COO^-$

$R^2 = -CH_2CH_2\overset{\oplus}{C}HNH_3COO^-$

$\Delta H^\circ_{(13)}$† was found to be −12.6 kcal. (*167*). Estimating the entropy change allowed the calculation of a value for $\Delta F^\circ_{(13)}$ of −7.0 kcal.‡ Therefore, $\Delta F'_{(13)}$ is −16.5 kcal., a markedly exergonic reaction.

The second reaction studied calorimetrically (*168*) was:

$$(CH_3)_3\overset{\oplus}{N}R^1 + HSR^2 \rightarrow (CH_3)_2H\overset{\oplus}{N}R^1 + CH_3SR^2 \qquad (14)$$

$\Delta H^\circ_{(14)}$ was found to be −8.6 kcal., from which $\Delta F^\circ_{(14)}$ was calculated to be −7.0 kcal. when the entropy change was estimated. Since Reaction 14 as written does not liberate a proton, the free energy change is independent of the pH of the standard state.

In order to estimate the relative energy levels of quaternary ammonium and sulfonium compounds, Reaction 15 must be introduced:

$$(CH_3)_2H\overset{\oplus}{N}R^1 \rightleftarrows (CH_3)_2NR^1 + H\oplus \qquad (15)$$

The pK_a of this reaction has been determined to be 9.5 (*168*); *ergo*, $\Delta F^\circ_{(15)} = +12.9$ kcal. Equation 16 may now be derived by subtracting Eqs. 14 and 15 from Eq. 13:

$$(CH_3)_2\overset{\oplus}{S}R^1 + (CH_3)_2NR^1 \rightleftarrows CH_3SR^1 + (CH_3)_3\overset{\oplus}{N}R^1 \qquad (16)$$

By addition of the corresponding free energies

$$\Delta F^\circ_{(16)} = \Delta F^\circ_{(13)} - \Delta F^\circ_{(14)} - \Delta F^\circ_{(15)}$$

the value of $\Delta F^\circ_{(16)}$ is found to be approximately −13 kcal. Thus the quaternary ammonium group is very much more stable than the strictly analogous sulfonium group. This is not unexpected, since tertiary amines are much stronger bases than thioethers.

In order to estimate the free energy of hydrolysis of the methyl-sulfonium bond of dimethylacetothetin or the methyl-ammonium bond

† The superscript ° on molar enthalpy and free energy changes indicates a standard state defined with all reactants and products at a concentration of 1 *M* in aqueous solution, with water in the pure liquid state, and at a temperature of 25°; the superscript ′ refers to the same standard state except that hydronium ion, if it partakes in the reaction, is at a concentration of 10^{-7} *M*; thus these quantities represent the molar standard energy changes at pH 7.

‡ This estimate of $\Delta F^\circ_{(13)}$ differs by 3.1 kcal. from the previously published estimate (*167*) due to a revision of the authors' estimate of the probable entropy change of the reaction. This revision was necessitated by subsequent determination of the entropy of ionization of dimethylglycine which was found to differ from that previously determined for glycine. Thus equating the entropy of ionization of methylmercaptoacetic acid to that of glycine was considered invalid, and more appropriate assumptions were made for the present calculation (*168*).

of betaine, an estimate of the free energy of hydrolysis of methionine is required (Eq. 17).

$$CH_3SR^2 + H_2O \rightleftarrows CH_3OH + HSR^2 \qquad (17)$$

The equilibrium constant for a similar reaction, the hydrolysis of S-adenosylhomocysteine to adenosine and homocysteine has recently been measured (*150*) and found to be about 10^{-6}; therefore ΔF° of that reaction is about +8 kcal. The synthesis of two other thioethers cystathionine (*169*) and S-methylcysteine (*170*) have been studied and found to be essentially irreversible, suggesting that the free energy change is of the same order as that observed for S-adenosylhomocysteine. Therefore it seems reasonable to assume that $\Delta F^\circ_{(17)} = +8$ kcal. Combining Eqs. 13 and 17 gives Eq. 18:

$$(CH_3)_2\overset{\oplus}{S}R^1 + H_2O \rightleftarrows CH_3SR^1 + CH_3OH + H\oplus \qquad (18)$$
$$\Delta F'_{(18)} = -8.5 \text{ kcal.}$$

Similarly, combining Eqs. 14 and 17 gives Eq. 19:

$$(CH_3)_3\overset{\oplus}{N}R^1 + H_2O \rightarrow (CH_3)_2\overset{\oplus}{N}HR^1 + CH_3OH \qquad (19)$$
$$\Delta F'_{(19)} = +1 \text{ kcal.}$$

It should be noted that these calculations suggest that the free energy of hydrolysis of a methyl-sulfonium linkage is in the same order as that of the pyrophosphate linkages of ATP (*171, 172*).

If one assumes that the hydrolysis of the methyl-sulfonium bond of S-adenosylmethionine is energetically equivalent to the hydrolysis of the same bond in dimethylacetothetin, it is possible to estimate the free energy change of the synthesis of S-adenosylmethionine:

$$ATP + CH_3SR^2 \rightarrow S\text{-Adenosylmethionine} + P_i + PP \qquad (20)$$
$$\Delta F'_{(20)} = -3 \text{ kcal.}$$

Even though the free energy change is small, reversibility of this reaction would be difficult to demonstrate in the dilute solutions used for enzymatic experiments, because of the increase in mole number. Even so, it might be expected that trapping experiments and isotope exchange experiments would have indicated reversibility of Reaction 20 and it is not immediately apparent why these have failed (*173*).

It is also of interest to consider the energy level of heteroaromatic onium compounds, particularly *N'*-methylnicotinamide. Since nicotinamide is a stronger acid ($pK_a = 5$) than dimethylglycine ($pK_a = 9.5$), but a weaker acid than a thioether, it seems reasonable to assume that the free energy of hydrolysis of the methylammonium bond of *N'*-methyl-

nicotinamide lies between the values calculated for betaine and its sulfur analog above: a value of —4 or —5 kcal. seems appropriate. This would agree with the observation that the synthesis of *N'*-methylnicotinamide from S-adenosylmethionine and nicotinamide is apparently irreversible (*174*). However, this figure differs appreciably from the value of —9 kcal. (*166, 175*) for the free energy of hydrolysis of nicotinamide riboside. It seems unlikely that there is such a large difference in the energy of hydrolysis of these analogous compounds. One possibility is that the above argument for estimating the energy of hydrolysis of *N'*-methylnicotinamide is invalid. Another possibility is that the energy of hydrolysis of methionine differs appreciably from that of S-adenosylhomocysteine. If it were, for example, 4 kcal. instead of 8 kcal., the value for $\Delta F'_{(18)}$ would be —12.5 kcal., for $\Delta F'_{(17)}$ would be —3 kcal., and the estimated value for *N'*-methylnicotinamide would be brought in line with that for nicotinamide riboside. It can be shown, however, that such a change in the estimated energy value for methionine hydrolysis would have no effect on the calculation of $\Delta F'_{(20)}$, which gives the free energy of enzymatic synthesis of S-adenosylmethionine.

In conclusion, it appears that the above considerations confirm and extend the earlier hypothesis that sulfonium compounds are energy-rich in the same sense as "high-energy phosphate," i.e., they have a high free energy of hydrolysis, in the order of —8 or —9 kcal. Quaternary ammonium compounds, on the other hand, are not energy-rich when one considers the free energy of hydrolysis. They can transfer their methyl groups to thiols, particularly homocysteine, by virtue of the "low-energy" level of thioethers, i.e., the positive energy of hydrolysis of thioethers. The difference in stability of these two types of methyl-onium compounds parallels the difference in basicity of thioethers and tertiary amines. The relative position of aromatic quaternary ammonium compounds is not definitely established, but it appears that they lie between sulfonium and aliphatic quaternary ammonium compounds, perhaps closer to the former.

B. Transfer Reactions from an Onium Pole

1. *Methyl Transfer from Dimethylacetothetin and Betaine*

An enzyme capable of transferring one of the methyl groups of dimethylacetothetin and/or betaine to homocysteine was first recognized by Borsook and Dubnoff (*55, 176*) in mammalian liver and kidney, and has been further studied by Durell, Anderson and Cantoni (*119*) in horse liver, by Maw (*120*) and by Fromm (*177*) in rat liver, by Sloane

et al. (*178*) in pigeon liver, and by Ericson in pig liver (*179*). The reaction catalyzed by this enzyme* is described by the equation:

Dimethylacetothetin + Homocysteine → Methylmercapto acid + Methionine + H^+ (21)

The most complete study of this enzyme appears to be that of Durell, Anderson, and Cantoni who have obtained from horse liver a preparation which may be considered essentially homogeneous as judged by physicochemical criteria (ultracentrifugation and electrophoresis). The purified enzyme has a molecular weight of approximately 200,000 and a turnover number of 3000 (μmoles of methionine/μmole enzyme/minute).

The value for the turnover number is considerably higher than that estimated for the other methylpherases so far studied. The enzyme is present in large amounts in horse liver and it may be calculated that it is equivalent to between 1 and 2% of the total liver protein. Although as noted above the enzyme has been found in the liver and kidney of several vertebrate species it has not yet been detected in microorganisms, protozoa, or plants.

Since a proton is formed in the reaction, the enzyme may be assayed manometrically (*120*), as is true also of other alkylpherase reactions in which the onium structure is lost (*180*).

The question of the relationship of the thetin-homocysteine methylpherase to betaine-homocysteine methylpherase is not entirely resolved. It is perhaps significant in this connection that dimethylthetin is not a naturally occurring compound. Furthermore it may be seen from Table VI, which describes the specificity of the purified thetin-homocysteine methylpherase for a variety of methyl donors, that betaine will serve as a methyl donor with this enzyme, albeit at approximately 1/200 the rate at which dimethylacetothetin is utilized. Originally Dubnoff and Borsook (*176*) showed that the enzymatic activities towards the two substrates could be distinguished by a difference in the stability of the two enzymatic activities at acid pH. It must be emphasized, however, that a *purified* preparation of betaine methylpherase free of activity towards thetin has not yet been obtained and that all preparations of the thetin enzyme can also utilize betaine as a substitute but at great sacrifice in activity. No evidence for the requirement of a cofactor has been conclusively disclosed either in the purified enzymes of horse and rat liver or in the

* Since enzymatic alkyl transfer reaction from onium compounds have common characteristics, it is suggested that uniform nomenclature be adopted and that the enzymes be identified by the donor compound, the acceptor compound, the group transferred, and the suffix *pherase* (e.g., dimethylacetothetin-homocysteine methylpherase).

TABLE VI

METHYL DONOR SPECIFICITY OF PURIFIED THETIN HOMOCYSTEINE METHYLPHERASE[a]

Substrate	Concentration (micromoles/ milliliter)	Time of incubation (minutes)	Methionine formed (micromoles/ milliliter)	Rate of methionine formation[b]
Dimethylacetothetin	30	30	1.3	420
Ethylmethylacetothetin	30	30	0.78	92
Dimethylpropiothetin	30	30	1.2	65
S-Methylmethionine	30	120	3.0[c]	1.2
Betaine	60	120	3.0	1.2
S-Adenosylmethionine	12	45	0	0
Acetylcholine	60	180	0	0
Choline	60	180	0	0
DL-Carnitine	60	180	0	0

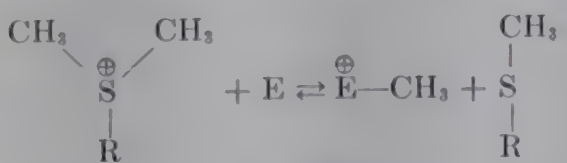
[a] From data by J. Durell, D. G. Anderson, and G. L. Cantoni, *Biochim. et Biophys. Acta* **26,** 270 (1957).

[b] Micromoles of methionine per hour per milligram of protein.

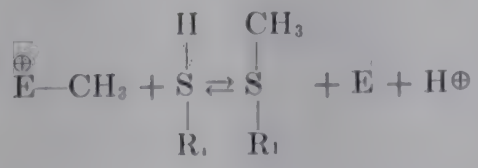
[c] This value represents 1/2 the measured value to correct for the fact that 2 equivalents of methionine are formed in this reaction. In all cases, DL-homocysteine was present at concentration of 0.010 *M*.

cruder pigeon liver enzyme, and reports by Ericson *et al.* (*181*) that a soluble cofactor is required have not been confirmed.

The reaction catalyzed by thetin homocysteine methylpherase has been considered as a model for transmethylation reactions in general and its molecular mechanisms have been studied in detail (*182*), in the hope that conclusions derived from this study might be applicable to other transalkylation reactions. The over-all reaction is known to be irreversible and recently the possibility that the reaction might proceed in a stepwise fashion, through a series of reversible bimolecular reactions (described in Eqs. 21a and 21b) involving the enzyme as a reactant, has also been ruled out experimentally.



(21a)



(21b)

where

$R = -CH_2-COO^-$

$R_1 = -CH_2-CH_2-CH(NH_3)-COO^-$

E = thetin-homocysteine methylpherase.

The sum of Eqs. 21a and 21b is identical with Eq. 21.

Since all attempts to demonstrate the formation of a methylated enzyme intermediate yielded negative results, it appears that the methyl group migrates from dimethylthetin to homocysteine directly when one or both substrates are bound to the enzyme. In the course of studies on

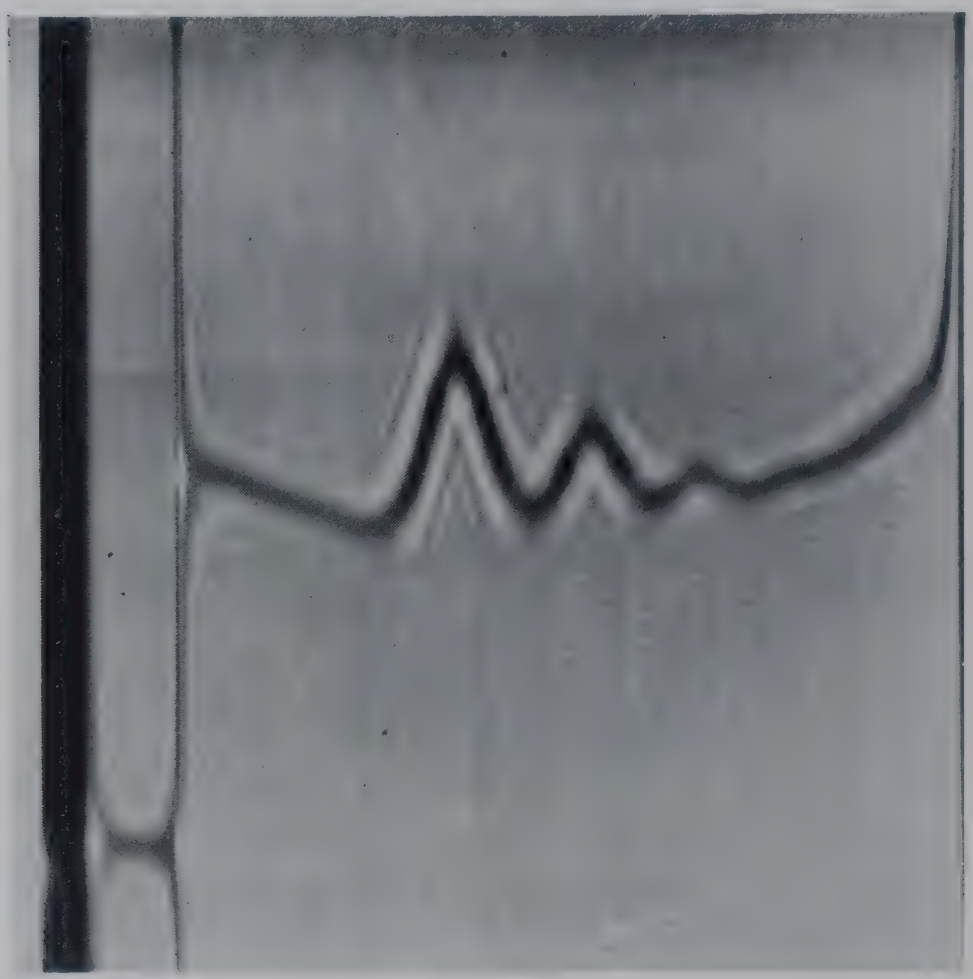


FIG. 15A

FIG. 15. Effect of mercaptoethylamine on sedimentation pattern of THMPase. (*A*) 10 mg. per milliliter of THMPase dialyzed 16 hours against 0.05 *M* potassium phosphate buffer, pH 7.4. (*B*) as in *A*, after treatment with 0.05 *M* MEA for 10 minutes at 37°. Both solutions were centrifuged in a Spinco analybest ultracentrifuge at 59.780 r.p.m. for 32 minutes. Bar angle 50 degrees. [Reproduced from *Biochim. et Biophys. Acta* **35,** 515 (1959).]

the physicochemical characteristics of the purified and electrophoretically homogeneous pherase from horse liver, it was discovered that the ultracentrifugal sedimentation pattern of the purified enzyme is complex, consisting of several components of which at least 4 are clearly visible (Fig. 15, A). However, treatment of the purified enzyme with any one of several thiols, e.g., homocysteine 3 mg. per milliliter at 37° for 10 minutes (Fig. 15, B) alters profoundly the sedimentation pattern, so

that now the purified enzyme sediments essentially as a homogeneous protein, with a sedimentation constant of 8.2 $S_{(20)}$. These data have been interpreted as indicating that the purified enzyme is essentially homogeneous but that it is not monodisperse because in solution it exists in a partially polymerized state as a mixture of different molecular species



FIG. 15B

representing the monomer, dimer, trimer, and higher polymers respectively. One feature of this phenomenon that is particularly intriguing is the fact that one of the substrates of the enzyme, homocysteine, is itself a sulfhydryl compound and as such capable of depolymerizing the enzyme. However, all the attempts to show a relationship between the catalytic activity of the protein and the polymerization reaction have led to the conclusion that the polymerization reaction does not involve a site, or sites, of the protein molecule which is, or are, active in the enzymatic catalysis.

As to the nature of the bonds linking the monomer units in the polymerized enzyme it has been suggested, on the basis of the data presently available, that the phenomenon depends upon the formation, and cleav-

age of intermolecular disulfide bonds. The possible biological significance of the polymerization reaction is at present obscure; it would be of the greatest interest to investigate enzymes with similar functions derived from sources other than horse liver to see if they would show similar physicochemical characteristics.

2. *Alkyl Transfer Reactions from S-Adenosylmethionine*

S-Adenosylmethionine is known to participate as a methyl donor in the enzymatic reaction involved in the synthesis of creatine (*183*) *N'*-methylnicotinamide (*174, 184*) choline from dimethylethanolamine (*17*), epinephrine from norepinephrine (*185*), 3-methoxyepinephrine from epinephrine (*186*), ergosterol [(*187*), see also reference *188*)] and 6-methylamino-2-hydroxyadenine (*189*). On the basis of these findings it may be suggested that S-adenosylmethionine is the actual methyl donor in all biological methylation reactions in which the methyl group is derived from methionine. However, it should be pointed out that there is no positive evidence to indicate that adenosylmethionine is actually involved in the numerous methylation reactions discovered in the plant kingdom, and an equally tenable supposition would postulate that S-methylmethionine or some other methyl-onium compound is the methyl donor.*

An important extension of the role originally envisaged for S-adenosylmethionine has been recently brought about by the discovery that S-adenosylmethionine, after decarboxylation, can act as a donor of its propylamine side chain to putrescine, and probably to spermine, for the biosynthesis of spermine and spermidine respectively (*190, 191*).

Although no metabolic or enzymatic reactions suggesting that adenosylmethionine might function also as an adenosyl donor have been discovered as yet, it is entirely reasonable to assume such a role for this compound. In this case, then, adenosylmethionine could act as a donor of any one of the three groups attached to the sulfonium center, depending on the specificities of the transferring enzymes and the acceptor molecule.

As noted above guanidinoacetate methylpherase is specific for one of the two stereoisomers of S-adenosyl-L-methionine, namely the (—)-S-adenosyl compound. Two other enzymes utilizing adenosylmethionine

* *Note added in proof*: Since this chapter was written Mudd (*192*) in the author's laboratory has conclusively shown that (—)S-adenosyl-L-methionine is the actual methyl donor in several plant transmethylation reactions. It may therefore be assumed that S-adenosylmethionine is the methyl donor in all biological methylation reactions in which the methyl group is derived from methionine. The biochemical role of S-methylmethionine still remains obscure.

as a substrate, catechol-*O*-methylpherase and the adenosylmethionine-cleaving enzyme of yeast, exhibit the same specificity for the steric configuration about the sulfonium center (*134*) (Table VII). Furthermore, it might be noted that the enzymes which synthesize the nucleoside, of which two have been studied in detail, possess similar steric requirements, as indicated by the fact that the optical rotation of S-adenosyl-L-methionine is identical whether it is formed by the methionine-activating enzyme of rabbit liver or of yeast.

TABLE VII

RELATIONSHIP BETWEEN THE CONFIGURATION OF *S*-ADENOSYLMETHIONINE AND ITS BIOLOGICAL UTILIZATION[a]

Substrate[b] configuration	Micromoles	Methylpherase	Per cent utilization
I	0.30	Guanidinoacetate	97
II	0.30	Guanidinoacetate	49
III	0.31	Guanidinoacetate	0
I	0.048	Catechol-*O*-methyl	100
II	0.051	Catechol-*O*-methyl	52
III	0.052	Catechol-*O*-methyl	0
I	2.06	Cleaving enzyme of yeast	100
II	4.12	Cleaving enzyme of yeast	47

[a] From data by G. de la Haba, G. A. Jamieson, S. H. Mudd, and H. H. Richards, *J. Am. Chem. Soc.* **81,** 3975 (1959).

[b] I = (−)-*S*-adenosyl-L-methionine, prepared enzymatically with methionine-activating enzyme of rabbit liver.

II = (±)-*S*-adenosyl-L-methionine, prepared by chemical methylation of *S*-adenosyl-L-homocysteine.

III = (+)-*S*-adenosyl-L-methionine, prepared from II by enzymatic resolution with guanidinoacetate methylpherase followed by reisolation.

By contrast thetin-homocysteine methylpherase from horse liver does not exhibit absolute optical specificity toward the sulfonium center inasmuch as 90% of a racemic substance, methylethyl acetothetin, was utilized as substrate.

Finally it is of interest to discuss another aspect of the steriospecificity of methylpherases, namely specificity with respect to the steric configuration about the α-carbon. Only one case has been studied, guanidinoacetate methylpherase which has recently been found to catalyze methyl transfer from (−)-S-adenosyl-D-methionine but at a rate considerably smaller than from the L-isomer (*134*).

The enzymatic reactions in which S-adenosylmethionine participates may be readily understood in terms of the chemical properties conferred on the molecule by the presence of a positively charged sulfur atom.

TABLE VIII

Alkyl acceptor	Product	Enzyme					
		Distribution	Purification		Requirements or cofactors	Specific activity (μmoles product/mg. protein/hour)	References
			Source	Fold			
Guanidinoacetate	Creatine	Mammalian livers only	Pig liver	80	SH	1.5	*150, 183*
Epinephrine and other catecholamines	3-methoxycatecholamines	Several tissues but not skeletal muscle	Rat liver	30	Mg^{2+}	0.8	*186*
Norepinephrine	Epinephrine	Bovine adrenal medulla extract	—	—	—	—	*185*
Unknown	Ergosterol	Yeast extracts	—	—	—	—	*187*
Dimethylethanolamine	Choline	Rat liver homogenate	—	—	—	—	*17*
2-Hydroxyadenine	2-Hydroxy-6-methylaminoadenine	*E. coli*					*189*
Putrescine	Spermine	*E. coli*	*E. coli*	50	None	0.5	*191*
Nicotinamide	*N'*-Methyl nicotinamide	Rat, pig, and dog liver	Rat liver	30	None	0.04	*174, 184*
S-Adenosyl methionine	2-Amino-4-butyrolactone	Bacteria, yeast	Yeast	20	—	0.3	*192, 193*

Thus, as noted in an earlier section, transalkylation reactions may be viewed as nucleophilic substitutions in which there is attack upon one of the carbons immediately adjacent to the positively charged sulfur. The over-all result is displacement of a thioether from this carbon atom synchronous with formation of a new bond between the same carbon atom and the attacking reagent, the "acceptor." In the present case, the nucleophilic attack may be spearheaded by a nitrogen, oxygen, or even carbon atom of the acceptor molecule, and it may be forecast, from what is known about transmethylation from other sulfonium compounds, that compounds containing a sulfur atom will be likewise found capable of attacking adenosylmethionine. In each case an enzyme specific for the acceptor compound has been found and significant comparative data are listed in Table VIII. It is interesting to note that the specific activities of the partially purified enzymes correlate pretty well with the rates that could be expected on the basis of the physiological significance of the different reactions.

In addition to the alkylpherase reactions discussed above adenosylmethionine is cleaved by yeast (*144*) and bacterial enzymes (*193*) to yield methylthioadenosine and 2-amino-4-butyrolactone. The mechanism of this reaction may be viewed as a special case of nucleophilic substitution, for in this case it is the carboxyl oxygen of adenosylmethionine itself which attacks the carbon atom adjacent to the positively charged sulfur leading to displacement of the thioether, methylthioadenosine, and to formation of the lactone. Alternatively, by consideration of the known tendency of onium compounds to undergo elimination reactions, and by analogy with the enzymatic cleavage of dimethylacetothetin to form acrylic acid and dimethyl sulfide (*111*), it has been suggested that the reaction might proceed with the formation of methylthioadenosine and elimination of an olefin, 2-amino-3-butenoic acid, which might then form 2-amino-4-butyrolactone by a lactonization reaction for which there is good biochemical precedent (*194*). Recently it has been shown experimentally (*195*) that with the yeast enzyme the enzymatic cleavage of S-adenosylmethionine proceeds directly to the butyrolactone, rather than with the formation of an olefin, thus establishing that the mechanism of this reaction is by nucleophilic attack.

3. *Alkyl Transfer and Base Exchange Reactions*

As suggested in earlier publications (*23*, *100*, *162*) transmethylation reactions may be considered as the simplest case of alkyl transfer reactions, for which the general formulation is Eq. 22.

$$R{-}CH_2{-}\overset{\oplus}{B} + H_2X \rightarrow R{-}CH_2{-}XH + B + H\oplus \qquad (22)$$

Where the onium compound is thiamine, DPN, or nicotinamide riboside, the enzymes involved while specific for the donor compounds, thiamine or DPN respectively, exhibit a broad substrate specificity for the acceptor molecule; furthermore they also are able to catalyze Reaction 23 which is known as a base exchange reaction.

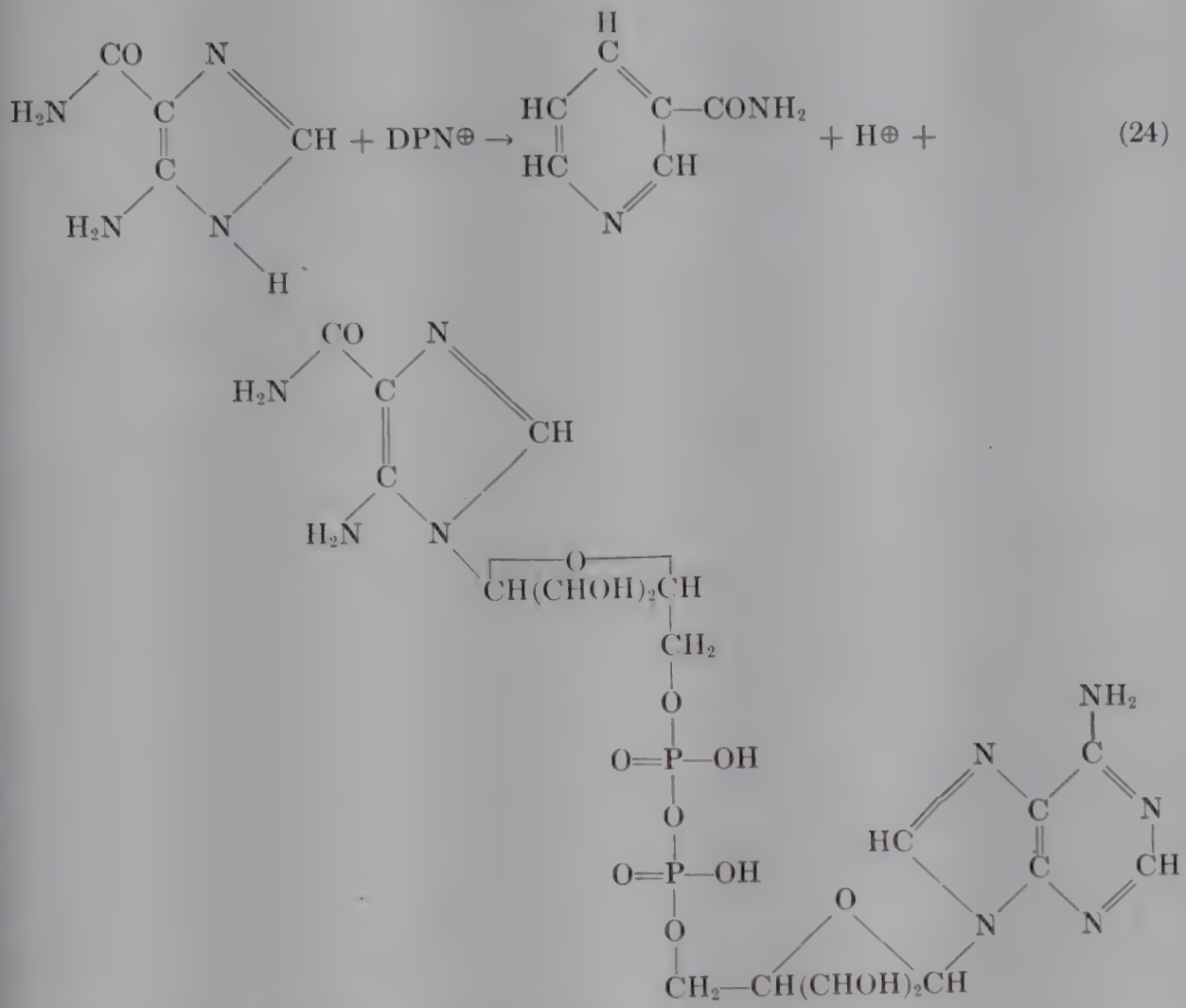
$$R—CH_2—\overset{\oplus}{B} + B' \qquad R—CH_2—\overset{\oplus}{B'} + B \tag{23}$$

Classical examples of such base exchange reactions are the exchange reactions between free nicotinamide and the nicotinamide moiety of DPN (*196*), and between free pyridine and the thiazole moiety of thiamine (*197*). Although a single onium-compound-specific enzyme may catalyze both the transalkylation and base exchange reactions, these reactions differ in two respects. In the first place, in the base exchange reaction the energy-rich onium bond is preserved and an onium compound with a different base is formed. Thus, for instance, in the exchanges catalyzed by a number of DPNases from various sources the nicotinamide moiety bound through a pyridinium linkage in DPN may be exchanged with another nicotinamide (*196*) or with a nicotinamide analog, such as acetyl pyridine, isonicotinic hydrazide, or others (*198*). Likewise in the thiaminase reaction enzymes from various sources, such as shellfish, fish liver, and bacteria, will catalyze the exchange of the thiazole moiety of thiamine with a pyridine moiety resulting in the formation of a pyridinium compound from a thiazolium and vice versa (*199*). On the other hand in the transalkylation reaction proper the onium bond is lost and H^+ is formed as for instance in the Reaction 24 which describes the synthesis of the 4-amino-5-carboxamideimidazole ribotide analog of DPN (*200*).

A second difference, which is in reality a consequence of the first, is the lack of reversibility of Reaction 22.

A further point requires clarification: generally enzymes catalyzing base exchange reactions are also capable of effecting a hydrolysis or phosphorolytic cleavage of the donor compound, that is to say they can utilize H_2O or orthophosphate (*194*) as the alkyl acceptor. Thus DPNases from various biological sources cleave DPN with the formation of free nicotinamide and ARPPR, which symbolizes the adenine ribose and phosphate moieties of DPN, and likewise the thiaminase of *Bacillus aneurinolyticus* catalyzes the hydrolytic splitting of thiamine into free thiazole and the hydroxymethyl pyridine moiety (*201*). All these observations can be unified by the formulation shown in Fig. 16 which is modeled after earlier schemes.

A more detailed analysis of the relationship between base exchange and hydrolytic, or phosphorolytic, reactions is possible in the case of



(24)

DPNase thanks to the important work of Grossman and Kaplan (*91, 201a*). This is of particular interest to comparative biochemistry since from their extensive studies of the reaction it became obvious that enzymatic cleavage of the ribosyl-pyridinium bond in nicotinamide riboside or DPN may or may not be associated with base exchange reaction depending on the source of the enzyme. Thus while spleen DPNase catalyzes both the base exchange reaction and the hydrolytic cleavage of DPN, DPNase from *Neurospora* and nicotinamide phosphorylase from human erythrocytes will catalyze only a hydrolytic, or phosphorolytic, reaction respectively. However, the ability to catalyze base exchange reaction can be conferred upon these latter enzymes by addition of a naturally occurring cofactor, which has been identified with ergothioneine (see Section II,A,4,*c*). An additional finding which emerged from these studies is the fact that base exchange in the presence of ergothioneine is always accompanied by formation of *N'*-methylnicotinamide and this may provide a clue as to the underlying molecular mechanisms.

The question of the reversibility of base exchange reactions is not

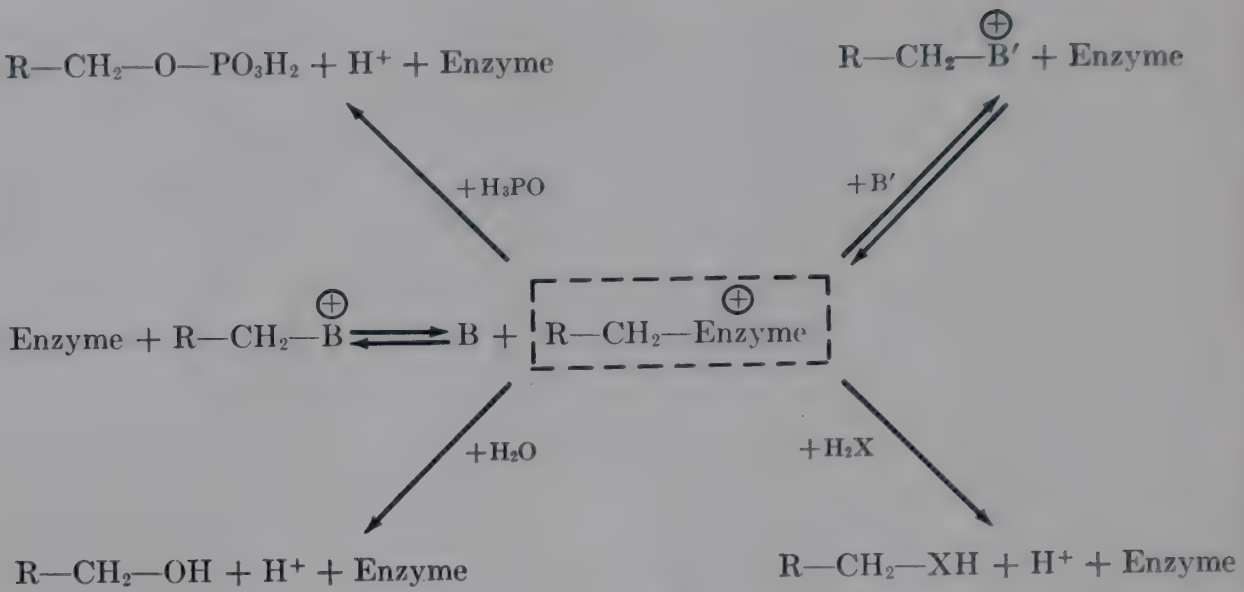
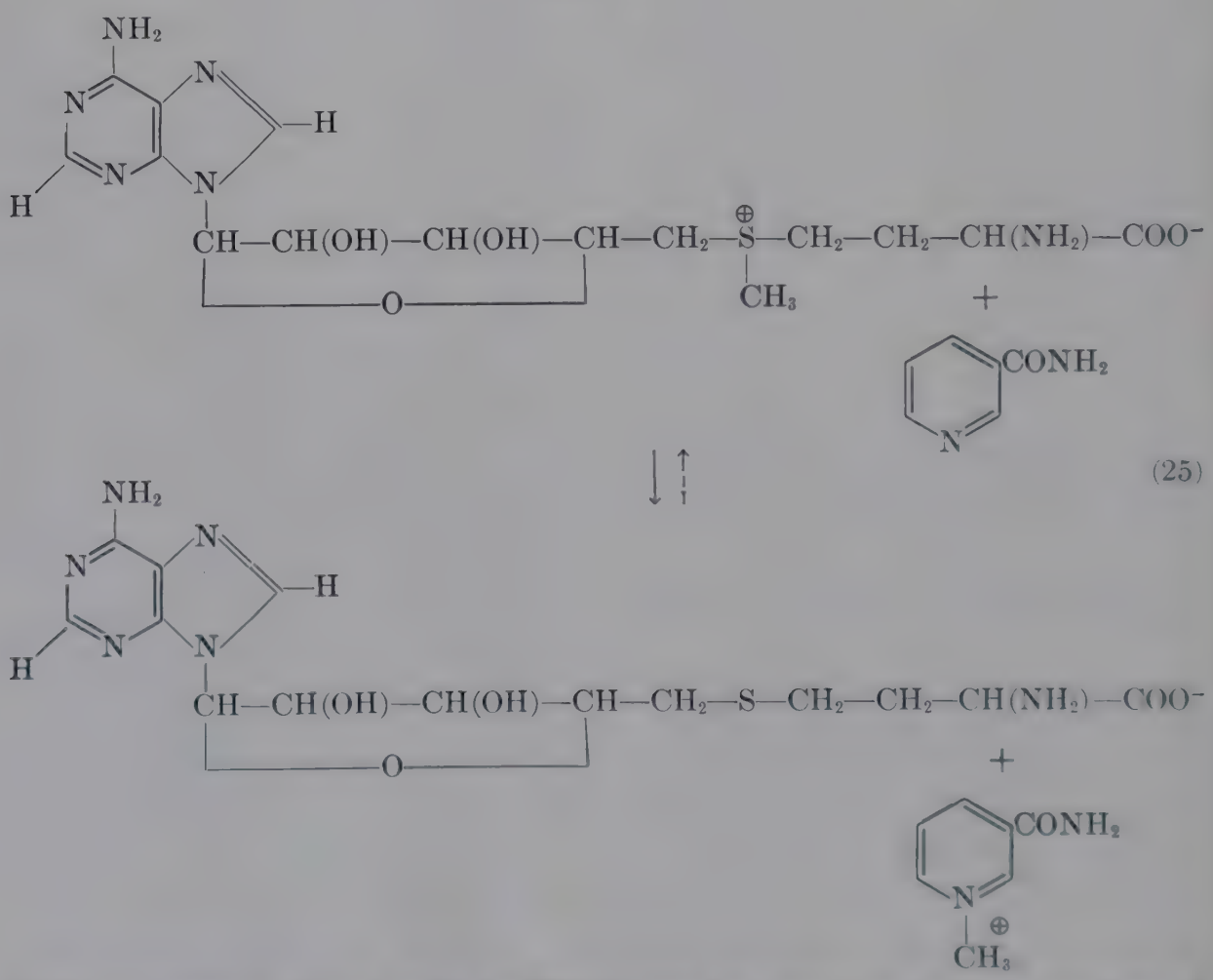


FIG. 16.



fully understood. Thus with the DPNases the reaction with a number of analogs of nicotinamide has been shown to be freely reversible (*196*, *198*); with the thiaminase some reversibility could be demonstrated from the pyridinium analogue but the equilibrium was very markedly in favor of thiamine cleavage (*199*). Finally base exchange between S-adenosylmethionine and nicotinamide as postulated in Reaction 25 could not be demonstrated (*174*).

Only future work will tell whether these differences are related to kinetic characteristics of the enzymes involved or to energetic factors involving the onium bond. It may be pointed out, however, that reversibility is easiest where an identical onium bond is formed (as in the nicotinamide exchange) and absent where the exchange reaction would require formation of an onium bond of a different type such as in the exchange postulated in Reaction 25 which would interconvert a sulfonium and a pyridinium bond.

C. Enzymatic Mechanisms in the Formation of the Onium Bond

Although onium compounds may be formed by the base exchange reaction discussed in the preceding section, it is evident that this mechanism cannot account for the biogenesis of the onium bond since it does not involve an actual increase in the number of onium compounds. It has become clear in recent years that phosphate bond energy may be utilized for the biosynthesis of the onium bond. Several examples for such a mechanism have come to light: (*a*) the formation of nicotinamide riboside from ribose-1-phosphate and nicotinamide and of nicotinic acid ribotide from nicotinic acid and 5-phosphoribose-1-pyrophosphate; (*b*) the formation of S-adenosylmethionine from methionine and ATP; and (*c*) the formation of thiamine from a phosphorylated pyrimidinehydroxymethyl moiety and thiazole.

1. *Biosynthesis of Pyridinium Compounds*

The formation of nicotinamide riboside is described in Eq. 26.

$$\overset{\oplus}{\mathrm{H}} + \text{Nicotinamide} + \text{Ribose-1-phosphate} \rightleftarrows \text{Nicotinamide riboside} + \text{Orthophosphate} \quad (26)$$

As noted above (*175*) the equilibrium constant of the reaction favors the back reaction, namely phosphorolysis of nicotinamide riboside. Perhaps, if this is a significant pathway for the biogenesis of nicotinamide riboside, the reaction may be pulled in the forward direction by coupling with any one of a number of reactions which utilize orthophosphate (e.g., oxidative phosphorylation, glycolysis, etc.). The cleavage of nicotinamide riboside has been discussed in the section on energetics of the onium bond, and in more detail by Kalckar (*166*). More recently evidence has accumulated to indicate that the synthesis of DPN proceeds from nicotinic acid by the following consecutive reactions (*202*).

$$\text{Nicotinic acid} + \text{5-Phosphoribose-1-pyrophosphate} \rightarrow \text{Desamidonicotinamide mononucleotide} + \text{Pyrophosphate} \quad (27)$$

$$\text{ATP} + \text{Desamidonicotinamide mononucleotide} \rightleftarrows \text{Desamido-DPN} + \text{Pyrophosphate} \quad (28)$$

$$\text{Desamido-DPN} + \text{Glutamine} + \text{ATP} \rightarrow \text{DPN} + \text{Glutamate} + \text{AMP} + \text{Pyrophosphate} \quad (29)$$

Only Reaction 27 is significant for the purpose of this discussion and it is clear from it that the ribosyl pyrophosphate bond is utilized for the synthesis of the onium bond of DPN.

2. *Biosynthesis of S-Adenosylmethionine*

The biosynthesis of S-adenosylmethionine has been investigated rather thoroughly particularly with the aim of elucidating the part played by ATP in the reaction and the mechanism underlying the energy utilization of the energy of a pyrophosphate bond for the synthesis of a sulfonium bond. The studies of Cantoni and Durell (*173*), and Mudd and Cantoni (*124*) on the methionine-activating enzyme of liver and yeast have revealed that the reaction proceeds as indicated by the equation

$$\underset{\alpha\ \beta\ \gamma}{\text{AR—P—P—P}} \xrightarrow{Mg^{2+}} \textit{S}\text{-Adenosylmethionine} + \text{Pyrophosphate} + P_i \qquad (30)$$

Furthermore it has become clear that the terminal or γ-phosphate of ATP is mineralized while the α- and β-phosphates give rise to pyrophosphate moiety. These results are of interest since they reveal an entirely new mechanism for the utilization of ATP in biological systems. Heretofore ATP has been known to act (*a*) as a phosphate donor with the concomitant formation of ADP, (*b*) as a pyrophosphate donor with the concomitant formation of AMP, and (*c*) as an adenylate donor with the resultant mineralization of its terminal pyrophosphate moiety. While the intimate mechanism of the reaction studied here is not yet fully understood, it is clear that this reaction cannot be grouped in any of the three categories mentioned above, and furthermore it is the first demonstration of the fact that ATP functions as an adenosine donor. The finding that pyrophosphate can be formed from the α- and β-phosphates of ATP is also new and indicates that the ribose-phosphate bond in ATP is not as inert as had been thought until now.

From the viewpoint of comparative biochemistry it is worthwhile to emphasize both the similarities and differences between methionine-activating enzyme of liver and yeast. Both enzymes require an unusually high concentration of Mg^{2+} for optimal activity. In addition, both proteins require a monovalent cation and the activity pattern is similar: NH_4^+, K^+, and Rb^+ are almost equally effective while Li^+, Cs^+, Na^+, and tris* are much less active or completely ineffective. Many enzymes which require a monovalent cation behave in a similar way, although differences exist in the detailed order of activities (*203–211*). The most striking difference between the two enzymes is the fact that

* Tris(hydroxymethyl)aminomethane.

the liver enzyme is very sensitive to fluoride ions exhibiting, for example, 28% inhibition at 8×10^{-4} *M* fluoride, while the yeast enzyme is not inhibited by fluoride at concentrations as high as 2×10^{-2} *M*. The liver enzyme is also more sensitive than the yeast enzyme to inhibition by one of the products of the reaction, pyrophosphate, although this difference is difficult to measure precisely because of the effect of pH upon the inhibition and the fact that different preparations of enzyme at the same stage of purification differ considerably in their sensitivity to pyrophosphate inhibition.

Finally while the liver enzyme shows a specific requirement for Mg^{2+}, the yeast enzyme responds when Mn^{2+} or other divalent cations replace Mg^{2+}.

3. *Biosynthesis of Thiamine*

Harris and Yavit (*212*) have shown that a cell-free extract of bakers' yeast will catalyze thiamine synthesis according to Reaction 30

$$\text{2-CH}_3\text{-4-NH}_2\text{-5-CH}_2\text{OPO}_3\text{H}_2\text{-pyrimidine} + \text{Thiazole} \rightarrow \text{Thiamine} + \text{P}_i \qquad (30)$$

whereas no thiamine synthesis takes place when the nonphosphorylated pyrimidine precursor is used. Other data also suggest that these extracts may catalyze the formation of the phosphorylated pyrimidine moiety, presumably through a reaction involving ATP. This reaction was postulated on the basis of earlier work (*213*) on the competitive inhibition of thiamine biosynthesis in *Neurospora* by the pyridoximers and vitamin B_6-like compounds. Although these data of Harris and Yavit are still preliminary, they may be accepted tentatively as evidence that phosphate bond energy is utilized for the formation of the thiazolium bond of thiamine.

4. *Others*

As outlined in Scheme 4 it is tempting to speculate that miokinine may be formed by condensation of trimethylamine and an unsaturated CoA derivative. Likewise the biosynthesis of dimethylpropiothetin could be as in Eq. 31.

$$H^+ + (CH_3)_2S + CH_2{=}CH{-}\overset{\overset{O}{\|}}{C}{-}CoA \rightarrow (CH_3)_2\overset{\oplus}{S}{-}CH_2{-}CH_2{-}COO^- + CoA \qquad (31)$$

Both reactions, if they did occur, would represent onium bond synthesis through utilization of acylmercaptan bond energy, and would be analogous to the biosynthesis of β-alanine from acrylyl-CoA and ammonia (*30*).

VI. Conclusions

Although much remains to be learned, it is becoming evident that onium compounds are an important group of naturally occurring compounds. The considerable range of their biological distribution, the chemical and enzymatic reactivity conferred by the presence of the onium pole, and the important physiological and pharmacological effects elicited by some of these compounds all point to the need for further explorations in the chemistry, biochemistry, and biology of these interesting compounds.

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References

1. G. W. Wheeland, "Advanced Organic Chemistry," 2nd ed. Wiley, New York, 1953.
2. G. M. Bennet and A. W. Chapman. *Ann. Repts. on Progr. Chem.* (*Chem. Soc., London*) p. 143 (1931).
3. P. B. D. de la Mare, *Ann. Repts. on Progr. Chem.* (*Chem. Soc., London*) p. 134 (1950).
4. M. L. Dhar, E. D. Hughes, C. R. Ingold, K. M. M. Mandour, G. A. Maw, and L. I. Woolf, *J. Chem. Soc.* p. 2093 (1948).
5. R. Willstätter, *Ber. deut. chem. Ges.* **35,** 584 (1902).
6. T. S. Stevens, E. M. Creighton, A. B. Gordon, and M. MacNicol, *J. Chem. Soc.* p. 3193 (1928).
7. T. Thompson and T. S. Stevens, *J. Chem. Soc.* p. 69 (1932).
8. C. R. Hansen and S. W. Kantor, *J. Am. Chem. Soc.* **73,** 4122 (1951).
9. A. J. Birch, R. J. English, R. A. Massy-Westoff, M. Slaytor, and H. Smith, *J. Chem. Soc.* p. 365 (1958).
10. M. Guggenheim, "Die Biogenen Amine." Karger, Basel, 1951.
11. P. J. G. Mann and J. H. Quastel, *Biochem. J.* **31,** 869 (1937).
12. H. A. Rothschild, O. Cori, and E. S. G. Barron, *J. Biol. Chem.* **208,** 717 (1954).
13. H. A. Rothschild and E. S. G. Barron, *J. Biol. Chem.* **209,** 511 (1954).
14. J. A. Stekol, S. Weiss, and E. I. Anderson, *J. Am. Chem. Soc.* **77,** 5192 (1955).
15. J. F. Nyc, *J. Biol. Chem.* **223,** 811 (1956).
16. W. Sakami and A. D. Welch, *J. Biol. Chem.* **187,** 379, (1950).
17. J. A. Stekol, E. I. Anderson, and S. Weiss, *J. Biol. Chem.* **233,** 425 (1958).
18. B. N. Ames, *J. Biol. Chem.* **226,** 583 (1957).
19. E. Adams, *J. Biol. Chem.* **217,** 325 (1956).

20. C. Yanofsky, *J. Biol. Chem.* **224,** 785 (1957).
21. B. N. Ames, *J. Biol. Chem.* **228,** 131 (1957).
22. E. Strack, P. Wözdehoff, E. Neubauer, and H. Geissendörfer, *Z. physiol. Chem.* **233,** 189 (1935).
23. G. L. Cantoni, *in* "Phosphorus Metabolism" (W. D. McElroy and B. Glass, eds.), Vol. 2, p. 129. Johns Hopkins Univ. Press, Baltimore, Maryland, 1952.
24. S. Ochoa, J. R. Stern, and M. C. Schneider, *J. Biol. Chem.* **193,** 691 (1951).
25. J. R. Stern, A. del Campillo, and I. Raw, *J. Biol. Chem.* **218,** 917 (1956).
26. W. Seubert and F. Lynen, *J. Am. Chem. Soc.* **75,** 2787 (1953).
27. D. E. Green, S. Mii, H. R. Mahler, and R. M. Bock, *J. Biol. Chem.* **207** (1954).
28. J. Gergely, P. Hele, and C. V. Ramakrishnan, *J. Biol. Chem.* **198,** 323 (1952).
28a. E. A. Hosein, private communication.
29. H. Ackermann, *Z. Biol.* **59,** 433 (1912).
30. E. R. Stadtman, *J. Am. Chem. Soc.* **77,** 5765 (1955).
31. B. T. Cromwell, *Biochem. J.* **46,** 578 (1950).
32. G. N. Cohen, B. Nisman, and M. Raynaud, *Compt. rend.* **225,** 647 (1947).
33. B. P. Eddy, *Nature* **171,** 573 (1953).
34. G. L. Cantoni and D. G. Anderson, *J. Biol. Chem.* **222,** 171 (1956).
35. R. H. Hayward and T. C. Stadtman, personal communication (1959).
36. F. E. Dyer and A. J. Wood, *J. Fisheries Research Board Can.* **7,** 17 (1947).
37. C. H. Best, W. H. Griffith, R. S. Harris, W. S. Hartroff, C. C. Lucas, and J. F. Nyc, *in* "The Vitamins" (W. H. Sebrell, Jr., and R. S. Harris, eds.), Vol. 2, p. 2. Academic Press, New York, 1954.
38. W. Feldberg, *in* "Metabolism of the Nervous System" (D. Richter, ed.), p. 493. Pergamon Press, London, 1957.
39. D. Nachmansohn, *Harvey Lectures* **49,** 57 (1955).
40. V. Erspamer and O. Benat, *Science* **117,** 161 (1953).
41. J. Banister, V. P. Whittaker, and S. Wijesundera, *J. Physiol.* **121,** 55 (1953).
42. V. P. Whittaker, *Biochem. J.* **71,** 32 (1959).
43. D. Nachmansohn and I. B. Wilson, *Advances in Enzymol.* **12,** 259 (1951).
44. R. W. Robbins and F. Lipmann, *J. Biol. Chem.* **229,** 837 (1957).
45. F. Lipmann, *Science* **128,** 575 (1958).
45a. A. Kaji and W. D. McElroy, *Biochim. et Biophys. Acta* **30,** 190 (1958).
45b. A. Kaji and J. D. Gregory, *J. Biol. Chem.* **234,** 3007 (1959).
46. D. W. Woolley and W. H. Peterson, *J. Biol. Chem.* **122,** 213 (1937).
47. J. de Flines, *J. Am. Chem. Soc.* **77,** 1676 (1955).
48. B. Lindberg, *Acta. Chem. Scand.* **9,** 1323 (1955).
49. B. Lindberg, *Acta Chem. Scand.* **9,** 917 (1955).
50. B. T. Cromwell and S. D. Rennie, *Biochem. J.* **55,** 189 (1953).
51. V. Stanek, *Z. physiol Chem.* **48,** 334 (1906).
52. B. T. Cromwell and S. D. Rennie, *Nature* **171,** 79 (1953).
53. B. T. Cromwell and S. D. Rennie, *Biochem. J.* **58,** 318 (1954).
54. V. du Vigneaud, S. Simmonds, J. P. Chandler, and M. Cohn, *J. Biol. Chem.* **165,** 639 (1946).
55. H. Borsook and J. W. Dubnoff, *J. Biol. Chem.* **171,** 363 (1947).
56. T. C. Stadtman, personal communication (1959).
57. V. S. Gulewitsh and R. Krimberg, *Z. physiol. Chem.* **45,** 326 (1905).
58. F. Kutscher, *Z. physiol. Chem.* **49,** 484 (1905).

59. H. Carter, P. K. Bhattacharyya, K. R. Weidman, and G. Fraenkel, *Arch. Biochem. Biophys.* **38,** 405 (1952).
60. G. Fraenkel and S. Friedman, *Vitamins and Hormones* **15,** 73 (1957).
61. G. Fraenkel, *Arch. Biochem. Biophys.* **50,** 486 (1954).
62. S. Friedman and G. Fraenkel, *Arch. Biochem. Biophys.* **59,** 491 (1955).
63. I. B. Fritz, *Acta Physiol Scand.* **34,** 367 (1955).
64. E. A. Hosein and H. McLennan, *Nature* **183,** 328 (1959).
65. D. S. Bell, *Ann. Repts. on Progr. Chem. (Chem. Soc., London)* p. 284 (1956).
66. C. Tanret, *J. pharm. chim.* **30,** 145 (1909).
67. G. Barger and A. J. Ewins, *J. Chem. Soc.* **99,** 2336 (1911).
68. G. Hunter and B. A. Eagles, *J. Biol. Chem.* **65,** 623 (1925).
69. G. Hunter, *Biochem. J.* **22,** 41 (1928).
70. D. B. Melville, W. H. Horner, and R. Lubschez, *J. Biol. Chem.* **206,** 231 (1954).
71. T. Mann and E. Leone, *Biochem. J.* **53,** 140 (1953).
72. J. N. Ashley and C. R. Harington, *J. Chem. Soc.* p. 2586 (1930).
73. G. Hunter, *Biochem. J.* **48,** 265 (1951).
74. D. B. Melville and W. H. Horner, *J. Biol. Chem.* **202,** 187 (1953).
75. D. B. Melville and S. Eich, *J. Biol. Chem.* **218,** 647 (1956).
76. D. B. Melville, W. H. Horner, C. C. Otken, and M. L. Ludwig, *J. Biol. Chem.* **213,** 61 (1955).
77. D. B. Melville, C. C. Otken, and V. Kovalenko. *J. Biol. Chem.* **210,** 352 (1955).
78. D. B. Melville, D. S. Genghot, E. Inamine, and V. Kovalenko, *J. Biol. Chem.* **223,** 9 (1956).
79. D. B. Melville, S. Eich, and M. L. Ludwig, *J. Biol. Chem.* **224,** 871 (1957).
80. H. Heath and J. Wildy, *Biochem. J.* **64,** 212 (1956).
81. J. Wildy and H. Heath, *Biochem. J.* **65,** 8P (1956).
82. H. Heath and J. Wildy, *Biochem. J.* **68,** 407 (1958).
83. F. Kutscher, *Z. physiol. Chem.* **24,** 775 (1910).
84. C. Reuter, *Z. physiol. Chem.* **28,** 167 (1912).
85. J. B. Mackenzie and C. G. Mackenzie, *J. Biol. Chem.* **225,** 657 (1957).
86. I. W. Grote, *J. Biol. Chem.* **93,** 25 (1931).
87. A. Kjaer and K. Rubinstein, *Nature* **171,** 840 (1953).
88. H. Heath and G. Toennies, *Biochem. J.* **68,** 204 (1958).
89. L. Eldjarn and A. Pihl, personal communication (1959).
90. D. Yanasugondha and M. D. Appleman, *J. Bacteriol.* **74,** 381 (1957).
91. L. Grossman and N. O. Kaplan, *J. Biol. Chem.* **231,** 727 (1958).
92. M. Zalokar and V. W. Cochrane, *Am. J. Botany* **43,** 107 (1956).
93. K. Jahkns, *Ber. deut. chem. Ges.* **29,** 2065 (1896).
94. G. Wiehler and L. Marion, *J. Biol. Chem.* **231,** 799 (1958).
95. B. Kalckar, *Proc. 4th Intern. Congr. Biochem., Vienna* (1958).
96. W. E. Knox and W. I. Grossman, *J. Biol. Chem.* **168,** 363 (1947).
97. F. Kutscher and D. Ackermann, *Z. physiol. Chem.* **221,** 33 (1933).
98. B. Riegel, D. W. Stanger, D. M. Wikholm, J. D. Mold, and H. Sommer, *J. Biol. Chem.* **177,** 1 (1949).
99. E. Gasteiger, L. J. Gergen, and P. Haake, *Biol. Bull.* **109,** 345 (1955).
100. G. L. Cantoni, *in* "Metabolism of the Nervous System" (D. Richter, ed.), p. 481. Pergamon Press, London, 1957.

101. W. A. Perlzweig, M. L. C. Bernheim and F. Bernheim, *J. Biol. Chem.* **150,** 401 (1943).
102. G. L. Cantoni, *J. Biol. Chem.* **189,** 203 (1951).
103. C. J. Walters, R. R. Brown, M. Kaihara, and J. M. Price, *J. Biol. Chem.* **217,** 489 (1955).
104. K. H. Beyer, H. F. Russo, S. R. Gass, K. M. Wilhoyte, and A. A. Pitt, *Am. J. Physiol.* **160,** 311 (1950).
105. R. R. Brown and J. M. Price, *J. Biol. Chem.* **219,** 985 (1956).
106. G. Toennies, *J. Biol. Chem.* **132,** 455 (1940).
107. C. Neuberg and K. Grosser, *Centr. Physiol.* **19,** 316 (1905).
108. H. Abel, *Z. physiol. Chem.* **20,** 253 (1894).
109. F. Challenger and M. I. Simpson, *J. Chem. Soc.* p. 1591 (1948).
110. O. Holmberg, *Arkiv Kemi, Mineral. Geol.* **21B,** No. 7, 1 (1946).
111. G. L. Cantoni and D. G. Anderson, *J. Biol. Chem.* **222,** 171 (1956).
112. F. Challenger, personal communication (1956).
113. G. L. Cantoni, unpublished observations (1955).
114. Y. Cheng Lin, unpublished experiments quoted by F. Challenger and M. I. Simpson (*109*).
115. A. J. Haagen-Smit, J. G. Kirchner, C. L. Deasy, and A. N. Prater, *J. Am. Chem. Soc.* **67,** 1651 (1945).
116. A. D. Welch, *J. Nutrition* **40,** 113 (1950).
117. G. A. Maw and V. du Vigneaud, *J. Biol. Chem.* **176,** 1037 (1948).
118. G. L. Cantoni, *Proc. 3rd Intern. Congr. Biochem., Brussels* (1955).
119. J. Durell, D. G. Anderson, and G. L. Cantoni, *Biochim. et Biophys. Acta* **26,** 270 (1957).
120. G. A. Maw, *J. Biochem.* **70,** 168 (1958).
121. G. L. Cantoni, *J. Biol. Chem.* **204,** 403 (1953).
122. G. L. Cantoni, *J. Biol. Chem.* **189,** 745 (1951).
123. F. Schlenk and R. E. de Palma, *Arch. Biochem. Biophys.* **57,** 266 (1955).
124. S. H. Mudd and G. L. Cantoni, *J. Biol. Chem.* **231,** 481 (1958).
125. H. Tabor and S. M. Rosenthal, *J. Am. Chem. Soc.* **79,** 2978 (1957).
126. J. A. Schiff, Ph.D. thesis, University of Pennsylvania (1956).
127. G. Jamieson and G. L. Cantoni, unpublished observations (1958).
128. S. H. Mudd and G. L. Cantoni, *Nature* **180,** 1052 (1957).
128a. K. Schwartz, personal communication.
129. J. Baddiley, G. L. Cantoni, and G. A. Jamieson, *J. Chem. Soc.* p. 2662 (1953).
130. J. Baddiley and G. A. Jamieson, *J. Chem. Soc.* p. 4280 (1954).
131. W. J. Pope and S. J. Peachey, *J. Chem. Soc.* p. 1072 (1900).
132. M. P. Balfe, J. Kenyon, and H. Phillips, *J. Chem. Soc.* p. 2554 (1930).
133. J. Baddiley and G. A. Jamieson, *J. Chem. Soc.* p. 1085 (1955).
134. G. de la Haba, G. A. Jamieson, S. H. Mudd, and H. H. Richards, *J. Am. Chem. Soc.* **81,** 3975 (1959).
135. L. W. Parks and F. Schlenk, *J. Biol. Chem.* **230,** 2951 (1958).
136. F. Schlenk, *Proc. 4th Intern. Congr. Biochem., Vienna* (1958).
137. P. Mamalis, and H. N. Rydon, *Nature* **166,** 404 (1950).
138. P. D. Lawley, *J. Chem. Soc.* p. 290 (1957).
138a. D. Mandel and G. Dunham, *J. Biol. Chem.* **11,** 85 (1912).
138b. V. Suzuki, S. Odaki, and T. Mori, *Biochem. Z.* **154,** 278 (1924).
139. F. Schlenk and R. L. Smith, *J. Biol. Chem.* **204,** 27 (1953).

140. F. Weygand, R. Junk, and D. Leber, *Z. physiol. Chem.* **291,** (1952).
141. F. Schlenk and R. E. de Palma, *J. Biol. Chem.* **229,** 1037 (1957).
142. S. H. Mudd, unpublished observations (1957).
143. H. Tabor, S. M. Rosenthal, and C. W. Tabor, *J. Biol. Chem.* **233,** 907 (1958).
144. S. H. Mudd, *J. Biol. Chem.* **234,** 87 (1959).
145. S. K. Shapiro and A. N. Mather, *J. Biol. Chem.* **233,** 631 (1958).
146. G. Schmidt, K. Serdarian, L. M. Greenbaum, M. D. Hickey, and S. J. Thaunhauser, *Biochim. et Biophys. Acta* **20,** 135 (1956).
147. F. Schlenk and J. A. Tillotson, *J. Biol. Chem.* **206,** 687 (1954).
148. G. L. Cantoni and E. Scarano, *J. Am. Chem. Soc.* **76,** 4744 (1954).
149. J. Baddiley and G. A. Jamieson, *J. Chem. Soc.* pp. 5941, 1085 (1955).
150. G. de la Haba and G. L. Cantoni, *J. Biol. Chem.* **234,** 603 (1959).
151. R. A. McRorie, G. L. Sutherland, M. S. Lewis, A. D. Barbou, M. R. Glazener, and W. Shive, *J. Am. Chem. Soc.* **76,** 115 (1954).
152. R. A. McRorie, M. R. Glazener, C. B. Skinner, and W. Shive, *J. Biol. Chem.* **211,** 489 (1954).
153. F. Challenger and B. J. Hayward, *Chem. & Ind.* p. 729 (1954).
154. M. A. Bennett, *J. Biol. Chem.* **141,** 573 (1941).
155. F. P. Meyers and J. R. Porter, *J. Bacteriol.* **50,** 323 (1945).
156. S. K. Shapiro, *Biochim. et Biophys. Acta* **18,** 134 (1955).
157. S. K. Shapiro, *J. Bacteriol.* **72,** 730 (1956).
158. J. K. Miettinen, S. Kari, T. Moisio, M. Aifthan, and A. I. Virtanen, *Suomen Kemistilehti* **B2,** 26 (1953).
159. L. Fowden, *Biochem. J.* **64,** 323 (1956).
160. A. Virtanen and P. Linko, *Acta Chem. Scand.* **9,** 551 (1955).
161. M. Kitagara, *J. Biochem. (Tokyo)* **25,** 23 (1937).
162. D. W. Wooley, *Nature* **171,** 323 (1953).
163. J. R. Catch, T. G. S. Jones, and S. Wilkinson, *Ann. N. Y. Acad. Sci.* **51,** 917 (1949).
164. L. Fowden, *Biol. Revs. Cambridge Phil. Soc.* **33,** 393 (1958).
165. A. Fujita, *Advances in Enzymol.* **20,** 389 (1954).
166. H. Kalckar, *in* "The Mechanism of Enzyme Action" (W. D. McElroy and B. Glass, eds.), p. 675. Johns Hopkins, Baltimore, 1954.
167. J. Durell and J. M. Sturtevant, *Biochim. et Biophys. Acta* **26,** 282 (1957).
168. J. Durell and J. M. Sturtevant, personal communication (1958).
169. F. Binkley, *J. Biol. Chem.* **191,** 531 (1951).
170. E. C. Wolff and S. Black, *J. Am. Chem. Soc.* **78,** 5958 (1956).
171. T. H. Benzinger and R. Hems, *Proc. Natl. Acad. Sci. U. S.* **42,** 896 (1956).
172. E. A. Robbins and P. D. Boyer, *J. Biol. Chem.* **224,** 121 (1957).
173. G. L. Cantoni and J. Durell, *J. Biol. Chem.* **295,** 1033 (1957).
174. G. L. Cantoni, unpublished observations (1953).
175. V. W. Rowen and A. Kornberg, *J. Biol. Chem.* **193,** 497 (1951).
176. J. W. Dubnoff and H. Borsook, *J. Biol. Chem.* **176,** 789 (1948).
177. H. J. Fromm, personal communication (1958).
178. N. H. Sloane, E. M. Boggiano, B. Carlowick, and B. L. Hutchings, *Federation Proc.* **15,** 357 (1956).
179. L. E. Ericson, *Acta Chem. Scand.* **12,** 1541 (1958).
180. R. H. Kenten, *Biochem. J.* **69,** 439 (1958).
181. L. E. Ericson, J. N. Williams, and C. A. Elvehjem, *J. Biol. Chem.* **212,** 537 (1955).

182. J. Durell and G. L. Cantoni, *Biochim. et Biophys. Acta* **35,** 515 (1959).
183. G. L. Cantoni and P. J. Vignos, Jr., *J. Biol. Chem.* **209,** 647 (1954).
184. G. L. Cantoni, *J. Biol. Chem.* **189,** 203 (1951).
185. N. Kirshner and McC. Goodall, *Biochim. et Biophys. Acta* **24,** 668 (1957).
186. J. Axelrod and R. Tomchick, *J. Biol. Chem.* **233,** 702 (1958).
187. L. W. Parks, *J. Am. Chem. Soc.* **80,** 2023 (1958).
188. G. J. Alexander and E. Schwenk, *J. Biol. Chem.* **232,** 611 (1958).
189. C. N. Remy, *Federation Proc.* **17,** 295 (1958).
190. H. Tabor, S. M. Rosenthal, and C. W. Tabor, *J. Biol. Chem.* **233,** 907 (1958).
191. C. W. Tabor, personal communication (1959).
192. S. H. Mudd, *Biochim. et Biophys. Acta.* **37,** 164 (1960).
193. S. K. Shapiro and A. N. Mather, *J. Biol. Chem.* **233,** 631 (1958).
194. W. R. Sistrom and R. Y. Stanier, *J. Biol. Chem.* **210,** 821 (1954).
195. S. H. Mudd, *J. Biol. Chem.* **234,** 1784 (1959).
196. L. J. Zatman, N. O. Kaplan, and S. P. Colowick, *J. Biol. Chem.* **200,** 197 (1953).
197. A. Fujita, Y. Nose, K. U. Hasegawa, and E. Hasegawa, *J. Biol. Chem.* **196,** 297 (1952).
198. L. J. Zatman, N. O. Kaplan, S. P. Colowick, and M. M. Ciotti, *J. Biol. Chem.* **209,** 467 (1954).
199. A. Fujita, Y. Nose, S. Uyeo, and J. Koizumi, *J. Biol. Chem.* **196,** 313 (1952).
200. S. G. A. Alivisatos and D. W. Woolley, *J. Biol. Chem.* **221,** 651 (1956).
201. A. Fujita and Y. Nose, *Vitamins (Kyoto)* **4,** 379 (1951).
201a. L. Grossman and N. O. Kaplan, *J. Biol. Chem.* **231,** 717 (1958).
202. J. Preiss and P. Handler, *J. Biol. Chem.* **233,** 493 (1958).
203. P. M. Nossal, *Biochem. J.* **49,** 407 (1951).
204. S. Black, *Arch. Biochem. Biophys.* **34,** 86 (1951).
205. E. R. Stadtman, *J. Biol. Chem.* **196,** 527 (1952).
206. W. K. Maas, *J. Biol. Chem.* **198,** 23 (1952).
207. H. G. Hers, *Biochim. et Biophys. Acta* **8,** 424 (1952).
208. J. F. Kachmar and P. D. Boyer, *J. Biol. Chem.* **200,** 669 (1953).
209. R. W. Von Korff, *J. Biol. Chem.* **203,** 265 (1953).
210. F. C. Happold and A. Struyvenberg, *Biochem. J.* **58,** 379 (1954).
211. R. E. Parks, Jr., E. Ben-Gershom, and H. A. Lardy, *J. Biol. Chem.* **227,** 231 (1957).
212. D. L. Harris and J. Yavit, *Federation Proc.* **16,** 192 (1957).
213. D. L. Harris, *Arch. Biochem. Biophys.* **60,** 35 (1956).

CHAPTER 6

Phototropism and Phototaxis

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I. Introductory

Movement towards or away from light is one of the most widespread reactions in living organisms. In higher animals the stimulus is perceived by the eye, whence it is conducted to the brain, and the resulting nerve current activates specific muscles. In some lower animals like planarians, however, and in some insect larvae, the perception of light is not limited to the eyes; for instance, blinding of the amphipod *Caprella* still allows some light-directed movement (*1*). Furthermore, blind worms, lamellibranchs, and many simpler creatures like hydroids and even protozoa react sharply to light. In some of these cases, as also in the toads (see Laurens, 2), it is the skin which is probably the photo-

sensitive organ ("dermatoptic sense"). The green flagellates and the zoospores of multicellular algae, which are classical objects for the study of movements initiated and directed by light, probably perceive light wholly or mainly by means of a photosensitive spot at the base of the flagella, or perhaps by an object called the stigma, a reddish spot near the anterior end and suggestively close to the base of the flagellum which causes the movement [see the excellent discussion of the evidence for this by Halldal (3)]. Light sensitivity is even found in the purple bacteria, but whether perception in them is limited to a particular spot or area is not known and indeed there is good evidence that the light-directed movement here is a function of the rate of photosynthesis (see Section VI, A).

In all these organisms the response to light is a free movement of the whole body directed towards or away from the light, and this is defined as *phototaxis*. In fungi and higher plants, as also in the colonial hydroids, the body is anchored at one end and the response to light is shown by a curvature. It is this which is *phototropism* properly speaking, and because its analysis has gone further in recent years than that of phototaxis the bulk of this chapter will be devoted to it. Zoologists have, it is true, used the term phototropism for free movements of animals, while botanists have been more precise in preserving the distinction, but the distinction is a valuable and even essential one to make, since (regardless of the means of detecting the light) the response to it is effected in an entirely different way in the two cases. For in Phototaxis the light influences the *organs of movement*, muscles, flagella and the like, while in Phototropism it influences the *growth* of the organism.

Experiments and observations on light-directed movements have a long history. That plants grow towards the light must have been known to horticulturists centuries ago, and was discussed by Ray in 1693 and by Hales in 1727. In modern science it was Réaumur in 1740 who first recorded that insects are attracted by light, while Trembley in 1744 made comparable observations in hydroids.

It was indeed Trembley, a noted contemporary of Réaumur, who carried out what was almost certainly the first experimental study of phototaxis (*4*). After putting a number of hydras into a glass of water on the table near the window, "Je remarquai un jour," he says, "qu'un grand nombre de Polypes étoit rassemblé sur le côté du verre tourné vers le jour. Je fus d'abord curieux de savoir si ce fait n'étoit qu'accidentel, ou bien s'il resultoit d'un penchant marqué que les Polypes eussent pour l'endroit du verre le plus éclairé. Pour m'en assurer, je fis faire un demi tour au verre. Le grand nombre de Polypes se trouva alors

sur le côté le moins éclairé de ce verre, et le côté qui étoit tourné vers le jour n'en avoit que quelques uns. Il s'agissoit donc de voir, si le grand nombre de Polypes passeroit du côté le moins éclairé, sur le côté le plus éclairé. Le jour après avoir tourné le verre, je trouvai que le côté le moins éclairé, celui sur lequel j'avois laissé beaucoup de Polypes, en étoit presque entièrement dépeuplé. Ils étoient dispersés dans le verre, et en chemin pour se rendre sur le côté du verre le plus éclairé; car le lendemain j'y en trouvai déja plusieurs, et au bout de quelques jours, il y en eut autant qu'il y en avoit sur l'autre, avant que j'eusse fait faire un demi tour au verre. . . . Après avoir vu la même chose plusieurs fois, je fus convaincu que les Polypes avoient un penchant particulier pour l'endroit du verre le plus éclairé." Subsequently he made more elaborate experiments with a chevron-shaped slit in a cardboard box, always finding the polyps assembled in a chevron-shaped group opposite the slit. Furthermore, the effect could not be due to warmth, for if the slit were turned towards the stove they did not assemble there,—only if it were turned towards the window. Hence he concluded, "Il paroit donc certain que c'est en effet la lumière qui attire ces Animaux sur le côté du verre le plus éclairé." Trembley also recognized that this attraction towards light is a very general phenomenon, and instances flies, moths, butterflies, etc., being attracted by lamps on summer evenings, as well as Adriatic fishermen who go out to catch marine polyps at night with a bright light above the water. This latter practice is, of course, common in the Mediterranean to this day.

The middle of the 19th century saw an increasing number of experiments, especially on animals, but it was the extensive studies of Jacques Loeb, beginning in 1888 and continuing for nearly 30 years, which brought out most of the characteristics of the light responses of animals.

Meanwhile the beautiful experiments of Engelmann on phototaxis of purple bacteria were so advanced for their time, 1882–1888, that they lay neglected for many years. Later they were taken up first by Oltmanns and then, over a period of years, by Mast, working with both plants and animals. However, phototaxis has never had quite the intensive study it deserves.

In higher plants the early botanists had ascribed phototropic curvatures to heating or to increased loss of water on the lighted side, and it was Darwin, Rothert, and others in the late 19th century who made these naive explanations impossible by using very photosensitive seedlings which could be exposed to low light intensities for short durations. Finally Blaauw, in the period 1908–1918, with his careful calibration of the light energy and precise, extensive observations, did essentially

the same thing for plants as Loeb for animals, but with greater flair for physical measurements. Loeb's aim seems to have been mainly to show that "phototropism" (phototaxis in most cases) is subject to rather simple physical laws, a view which now appears to represent an oversimplification of the matter. The modern biophysical and biochemical approach has brought out the true complexities of the light-directed movements, and in the case of plants has led to some understanding of the intimate relation between phototropism and the control of growth.

II. "Phototropism" (the Phototactic Response) in Animals

Much of the work on phototaxis, especially on animals, was done in the early part of this century and has little or no biochemical content. The methods and approaches used were sometimes those of behavioral psychology and more often of simple nature study. For this reason no historical review will be given but the reader is referred to the books of Loeb (5) and Rose (6); the latter contains a series of extensive bibliographies of the older literature. Characteristic of the work is the large number of different animals employed and the scarcity of prolonged analytical studies with any single object selected as specially favorable. Another characteristic is the frequent lack of precise definition of the light stimulus, either in duration, intensity, or wavelength (although it was often defined in direction). Effects of temperature and humidity may often have influenced the experiments also, when bright lights were used for long exposures, especially on insects. In many instances two separate phenomena are involved, namely, orientation of the body and actual directed movement. The orientations in insects have been extensively studied (e.g., by Garrey (7)). In rotifers, orientation is determined by the light-detecting organs, while the photosensitivity of the skin determines movement (8). This participation of two processes makes the work still harder to evaluate, because of the numerous muscles involved in orientation and locomotion, the participation of the nervous system, and the role of adaptation and learning.

Nevertheless the work on animal phototaxis did bring out four important characteristics of the reaction, which have a wide (though not universal) generality and have some very close parallels in the phototropisms of plants.

(1) *Reversal of sign.* A large number of animals exhibit *positive* phototaxis, i.e., movement towards the light source, at low light intensities, but this changes at higher light intensities to *negative* phototaxis, i.e., movement away. Well-studied examples include the earthworm *Allolobophora*, the annelid worms *Serpula* and *Spirographis*, the nauplii of the barnacle *Balanus perforatus*, and a number of insects. The

zoospores of green algae, as well as the motile algae such as the unicellular *Chlamydomonas* and the colonial Volvocales, behave in the same way (see Section VI, A). The ecological result of this behavior is that small aquatic organisms often assemble near the edge of a region of shadow, e.g., the small crustacean *Caprella* (*1*). The intensity at which change of sign occurs has seldom been precisely determined; with *Allolobophora* in continuous white light it is at about 0.012 meter-candles (*9*). However, there is often marked variation in sensitivity within a population, and some *Balanus* nauplii may remain positive at intensities high enough to make most of the others negative. Furthermore, with *Balanus,* as also with the alga *Volvox* and doubtless with other organisms, the positively phototactic individuals become gradually negative on continuing exposure, as if the product of the light reaction were accumulating. This invites comparison with the proposed explanation for negative phototropic curvatures in *Avena* (see Section VII, C) based on irreversible bleaching of the photoreceptor. There can also be adaptive changes, light-adapted organisms becoming less sensitive. Mast (*10*) even found that light-adapted colonies of *Volvox* or *Pandorina* are sometimes positive in high intensities and negative in low ones.

(2) *Influence of Salts and Temperature.* The phototactic reaction, and particularly the change of sign, is influenced by several factors other than light. In marine copepods, annelid larvae, etc., dilution of the sea water makes positive animals become negative, and negative animals more strongly negative. Correspondingly, concentrating the sea water (by evaporation) tends to weaken positive reactions and strengthen negative ones. Individual salts have a host of effects (see Rose *6*). Isotonic NaCl stops the reaction altogether. CO_2 is also very active; negatively phototactic *Gammarus pulex*, as well as *Volvox*, is made positive by dissolved CO_2. This effect is probably due to pH change, since a variety of acids have a similar effect (*10*). The individual constituents of sea water, especially Ca and Mg, are very effective in determining reversal of sign (*10a*). Temperature is also effective; Loeb has described a number of cases in which, with the same light intensity, phototaxis is positive at low temperature, negative at high. These include *Daphnia*, *Limulus*, *Artemia*, *Temoria*, and *Polygordius*. However, the algae *Hematococcus*, *Volvox*, and *Pandorina* change in the opposite direction. There is some evidence also, with *Balanus*, that the rate at which sign-reversal takes place due to increased light intensity is itself temperature-sensitive.

(3) *Bunsen-Roscoe Law or Product Law.* One of the most frequent subjects of study has been the relation between the movement and the total light dosage, i.e., the product of the intensity and the duration of

exposure. Since this was first studied by Bunsen and Roscoe for photochemical reactions, the dependence of phototactic response on this product is often referred to as the Bunsen-Roscoe Law, or simply the $I \times t$ Law. The researches of Blaauw established this law for phototropism of plants, under certain conditions (see Section IV, A). Loeb and Wasteneys (*11*) tested it with the phototropic curvature of the hydroid *Eudendrium,* by counting the number of positive curvatures developing in the dark after a fixed exposure; they found it to hold for three intensities. Loeb and Northrop (*12*) used a method for phototaxis in which two lights are placed before the animals, one being obscured to a variable extent by a rotating sector-disc. The angle of movement of *Balanus* nauplii between the two light sources was then found to be proportional to the duration of illumination in the second source, provided that the ratio of illumination times was not greater than 10:1. Such experiments with continual intermittent exposure may not necessarily be compared with single exposures of varying duration like those used by Blaauw, since they may allow of recovery or reversion in the photosensitive system during the dark period (see, e.g., Dolley, *13*). Since Loeb, the validity of the $I \times t$ law has been established, though also within marked limitations, for a number of animals (see Rose *6*). In insects it appears to hold only for short exposures and low intensities (*14*). While the initial photochemical reaction is doubtless proportional to the total incident light energy ($I \times t$), so many steps must intervene between it and the resulting movement that it is probably naive to expect any close correspondence.

(4) *Spectral sensitivity.* Study of the sensitivity to wavelength is one of the best means of establishing the nature of the photoreceptor. Its use in vision is discussed in Chap. 7 in this volume. Unhappily, the technical difficulties involved in producing light beams of equal energy at different wavelengths, as well as the imperfections of many color filters, combine to render most of the early work on the spectral sensitivity of phototaxis open to serious doubt. However, a general insensitivity to the red has been noted in a variety of animals. Omitting a number of conflicting or improbable data, the following regions of maximum sensitivity seem to be established:

Yellow to yellow-green	*Balanus* larvae, *Euproctis* caterpillars
Blue-green	*Palaemonetes, Loligo*
503 mμ	Blowfly larvae
Blue	*Cyclops, Chydorus, Diaptomus,* and *Bufo* species (both eyes and skin), probably most amphibians
483 or 495 mμ	*Arenicola* larvae
483 mμ	*Lumbricus, Phacus*

The relative insensitivity of many animals to the ultraviolet is mainly due to the powerful absorption of the ultraviolet by the eye lenses, which act as cut-off filters in this region (*15*). The data as a whole suggest the action of several red to purplish light-absorbing systems, and it is evident that what is being measured is essentially the absorption spectra of a group of "visual purples" (see Chapter 7 by Wald, in this volume).

III. Phototropism in Plants

So much of the work on phototropism has been in fields remote from biochemistry that it hardly seems appropriate to present a complete historical review here. Instead, this presentation will be limited to those aspects of phototropism which have been treated biochemically or biophysically, or are at least capable of such treatment. An extensive review of early work on phototropism, along with some original material, was presented by du Buy and Nuernbergk in 1932–1935 (*16*). Briefer surveys have been given by van Overbeek (*17*), du Buy (*18*), Went and Thimann (*19*), and Oppenoorth (*20*), the last-named author adding much additional data on the part played by auxin. More recently the subject has been reviewed by Brauner (*21*), Went (*22*), and Galston (*23*).

A phototropic curvature results from a difference in growth on the two sides of an elongating organ. If the curvature is *positive*, i.e., towards the light, it could be due to: (a) deceleration of growth on the lighted side (or a greater deceleration there than on the shaded side); (b) acceleration of growth on the shaded side (or a greater acceleration there than on the lighted side); or (c) both. Corresponding possibilities are offered for *negative* curvatures. It follows, then, that measurements of growth rate play an important part in the interpretation. They may be made either on both sides, during the actual curvature, or while the organ is growing straight, under all-round illumination of the same intensity and duration as that causing curvature.

It stands to reason that the only part of an organ which can curve in response to light is the part which is elongating. Curvatures are therefore limited to the elongating portions of the seedlings, roots, stems, fungal hyphae, or other objects used for study. However, in some cases the limitation is more severe than this, and at least in many seedlings it is clear that only part of the growing zone is able to respond to light. There is also the possibility, which cannot be excluded, that the incidence of light may have brought about elongation in a region which had not been elongating in the dark. For these reasons growth measurements have to be made in detail, and by zones, in order to delimit the response as much as possible.

Ever since the experiments of Charles and Francis Darwin in 1880, extensive use has been made of rapidly elongating seedlings for phototropic studies. Such seedlings, especially of the grasses, are, as the Darwins showed, exceedingly sensitive to light. Obviously seedlings have the advantages that they can be had in large numbers and can be handled in a small space. But their main advantage is that they can be grown in the dark, the cotyledons supplying all nutrients, and they can then be illuminated under controlled conditions. The majority of the critical experiments have been done on such material. The important reservation has to be made, however, that if growth is to be measured before or after the light treatment, some kind of weak illumination must be supplied in order to make the measurements. There are, it is true, auxanometers such as those of de Bouter (*24*) and Idle (*25*) which operate in complete darkness, but many of the published measurements involve some light, and this in turn may have influenced the phototropic reaction. For instance, red light treatment one hour before phototropic exposure reduces the sensitivity of *Avena* coleoptiles to blue light by a factor of two or three times (*26*). In a few cases, isolated parts of plants have been made use of, and here too some light is in general necessary in order to carry out the surgical operations.

Other external influences may also modify the response to light. Nutrition, temperature, water supply, and the humidity of the air are obvious external factors which must in general be standardized for the best results. Among internal factors, two are of major importance: (a) pigmentation, which will not only influence light absorption directly, but which itself may be modified by preillumination and perhaps by other treatments; (b) the age of the plant and the accompanying variations in growth rate and in the distribution of growth zones.

In summary, experimental work on the phototropism of seedlings has six aspects: (1) the observation and measurement of the reaction itself; (2) the influence exerted on the reaction by external variables, including illumination before and afterwards; (3) the effects of internal factors; (4) the measurement and localization of growth responses associated with the reaction; (5) determination of the influence of the intensity, duration, and wavelength of the light; (6) the proposal of interpretations and hypotheses, and special experiments designed to test these proposals.

All the above applies equally well to roots as to shoots, but the phototropism of roots is generally much less marked than that of shoots. As a rule it is either absent or negative in sign, which has obvious ecological advantages for the growing plant. Mainly because of their relatively weak response, phototropism of roots has been little studied.

It has been shown, however, that the negative curvature is associated with an acceleration of growth by light (27).

Next to seedling shoots, the objects of study most used have been fungi. The mycelium growing more or less horizontally on nutrient medium may show some photosensitivity, but it commonly does not; what is exceedingly light-sensitive is the vertically growing sporangiophore or spore bearer. Those of *Pilobolus* and *Phycomyces* have been the most used. The former forcibly expels the spore mass when mature, which has led to its use in elegant demonstration experiments (28), but the latter is easier to culture under sterile conditions and has been more widely adopted for critical work. These sporangiophores differ, of course, from the seedlings in being unicellular or coenocytic, while the seedling is a tissue of many thousands of cells.

IV. Analysis of the Phototropic Reaction

A. The Two Types of Phototropism in *Avena* Coleoptiles

At present, biochemical interest in phototropism is centered on three main aspects: the role of auxins, the nature of the photoreceptors, and the biochemical mechanisms suggested by the various kinds of phototropic reactions. The phototropic behavior of the *Avena* (oat) seedling is by far the best described and includes most of the types of phototropism generally encountered; an outline of its responses is therefore essential before any biochemical interpretation can be offered.

Under natural conditions an *Avena* seedling will grow toward a source of white light. If this source is not on the line of the plant's axis, a phototropic curvature will result. The magnitude of this curvature is only determined by the original orientation of the plant axis with respect to the light, providing the light operates continuously and there are no physical obstructions. Thus, Darwin (29), observing the curvatures in a row of seedlings continuously exposed at various distances from a dim source of white light, noted that the response was not particularly graded with respect to intensity; i.e., curvatures in plants far from the source were not very different from those close to the source. Curvatures under continuous exposures begin to appear a half hour or so after the beginning of the exposure, near the tip of the coleoptile. They increase steadily until eventually the whole plant is bent toward the light in a smooth arc.

The early discoveries showed that a very dim source of white light would suffice for curvatures if the exposures were long. Czapek (30) found it necessary to expose seedlings to the light for at least seven minutes in order to obtain an observable reaction subsequently (7 min-

utes = "presentation time"). Similarly Ohno (*31*) found an 8-minute exposure necessary for a positive response to a certain lamp. On the other hand Blaauw (32) calculated from his own results that Ohno's lamp should give observable positive responses with exposures as short as 1/10 second, and, furthermore, Blaauw found a distinctly graded response in his system.

A requirement for more precise analysis is a defined stimulus, consisting of exposures for a fixed time to monochromatic light. In this case the complexity of the response is revealed, and it becomes clear that more than one reaction is involved.

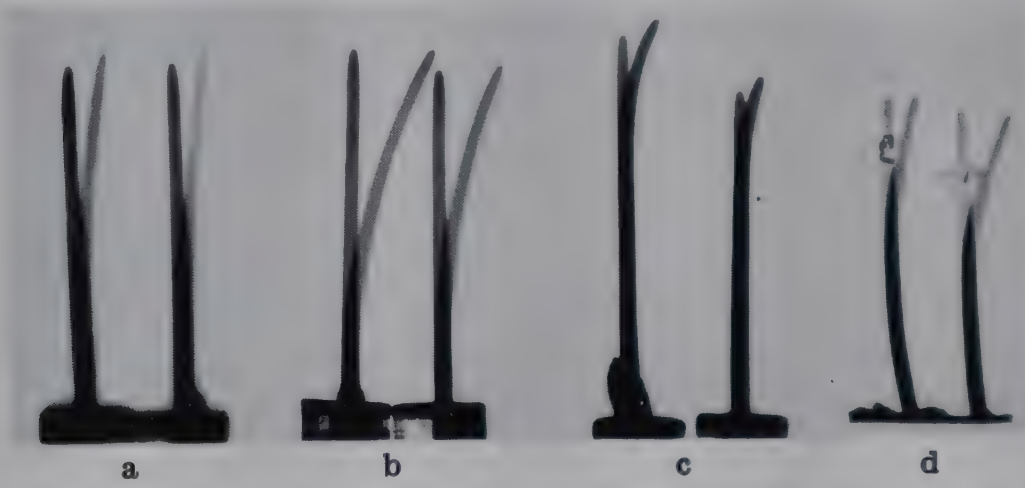


FIG. 1. Typical curvatures of *Avena* coleoptiles (var. Victory) caused by light and auxin. *a*, base response caused by 10 minutes of medium intensity blue light (436 mμ); *b*, base response caused by 10 seconds of ultraviolet light (254 mμ); *c*, tip response caused by 1 second of blue light of the same intensity and wavelength as in *a*; *d*, auxin curvature caused by application of a tip diffusate in agar to decapitated plants. (Direction of light from the right. All shadowgraphs taken at 0 and 90 minutes from treatment.)

With small amounts of light a "tip response" is observed; this begins to appear 20 or 30 minutes after the stimulus, develops continuously (for several hours, if the plant is placed on a clinostat), and extends in a smooth arc from the tip basally. The length of the curved zone depends on the development time, because the curvature migrates steadily downwards. Such curvatures are very similar in appearance to those produced by one-sided application of auxin to a decapitated coleoptile (see Fig. 1, *c* and *d*).

Protracted stimuli produce what has been called a "base response." The coleoptile begins to bend, along the entire length exposed, 10 or 20 minutes after the stimulus (33) (Fig. 1, *b*). For limited stimuli these responses are small; the upper parts of the coleoptile begin to straighten out after an hour, so that after 90 minutes the coleoptile looks quite straight, but tilted slightly in the direction of the stimulus (Fig. 1, *a*).

These two responses are only distinct and separable under certain exposure conditions. In Table I the responses to a wide variety of stimuli, using monochromatic blue light, are tabulated in a simple (and approximate) way; the data are collected from a number of separate experiments. The necessity for specifying both intensity and duration of stimulus is apparent from the results.

TABLE I

GENERAL SUMMARY OF THE PHOTOTROPIC RESPONSES OF *Avena* TO MONOCHROMATIC BLUE LIGHT

I Intensity ergs/cm.^{2} sec blue (436 mμ)	II Stimulus duration (seconds)	III Dose ergs/cm.^{2} (= I × II)	IV Curvature (degrees) observed 90 minutes after stimulus	V Type of response
1	1	1	+ 3[a]	tip
10	1	10	+12	tip
1	10	10	+12	tip
10	10	100	+25	tip
1	100	100	+25	tip
100	10	10^3	0	
10	100	10^3	0	
1	1000	10^3	+10	tip and base
500	10	5×10^3	− 8	tip
100	50	5×10^3	− 4	tip
5	1000	5×10^3	+ 8	base (+ tip?)
100	100	10^4	0	
10	1000	10^4	+ 8	base (+ tip?)
2	continuous (5400)	10^4	+40	tip + base
1000	100	10^5	0	
100	1000	10^5	+ 8	base
20	continuous (5400)	10^5	+60	tip + base
1000	1000	10^6	+ 8	base
1000	continuous (5400)	5×10^6	+20	base + tip

[a] Just observable.

Figure 2 (*34*) shows the response curves for energy applied at two wavelengths, 436 and 365 mμ; each point is the average of 10 to 14 individual curvatures. Above a light dosage of 10^4 ergs/cm.^{2} exposure times greater than 100 seconds were required, in which case base curvatures begin to appear. It seems quite possible that Czapek and Ohno may have applied doses greater than 10^3 ergs/cm.^{2}, whereas Blaauw did not, accounting for the amazing discrepancy in the presentation time determinations.

Results such as those in Table I and Fig. 1 have led to the rather wide adoption, for the *Avena* coleoptile, of the terms "first positive" and "second positive" curvature, in line with the idea that the phototropic process is a periodic function of the light dose. Thus, the curvatures observed 90 minutes after exposure are usually positive for low doses, negligible (indifferent) for intermediate doses, and finally positive again

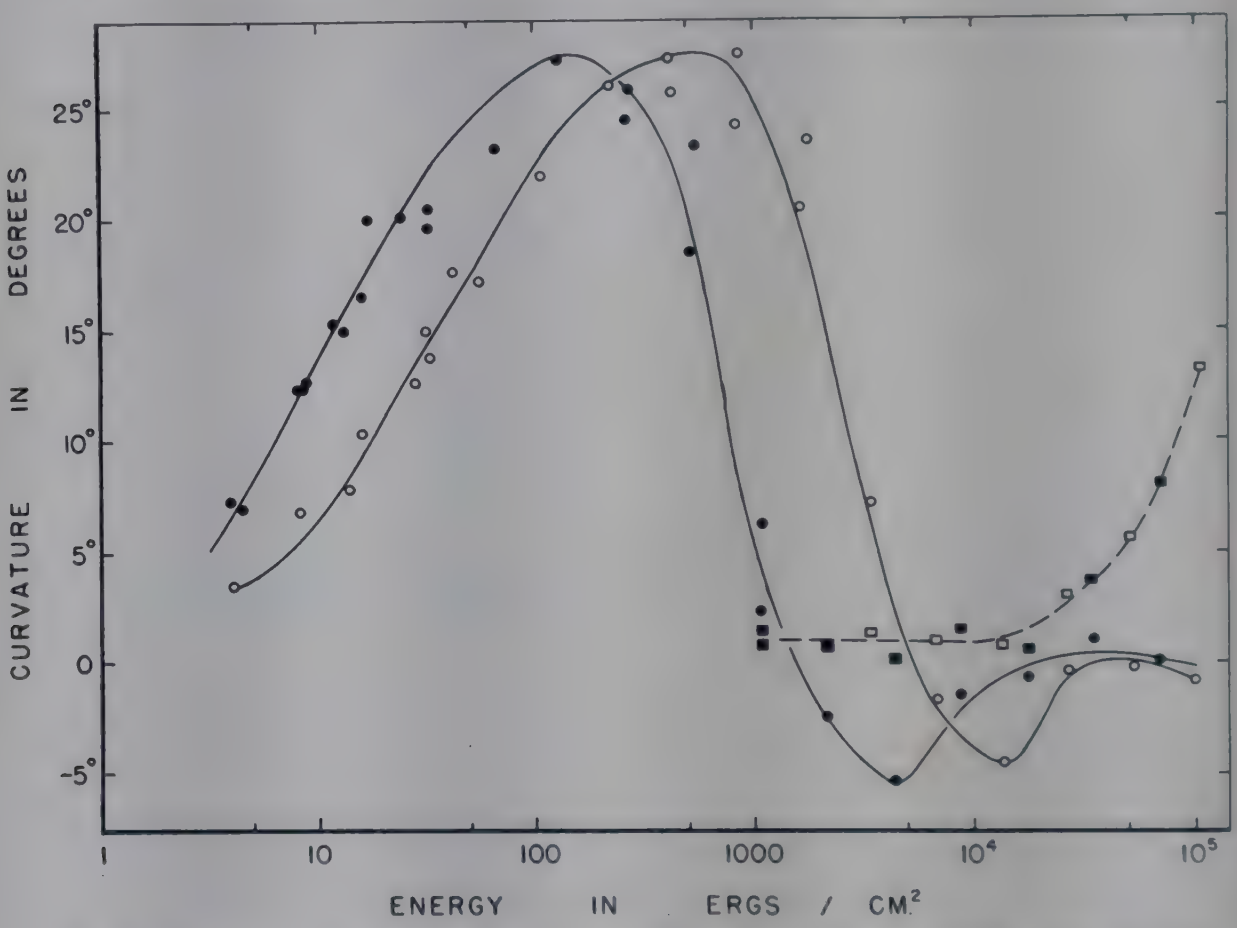


FIG. 2. Response curves at two wavelengths. Ordinates: curvature after 90 minutes; abscissa: total light dose. KEY: ●, 436 mμ, tip curvature; ■, 436 mμ, base curvature; ○, 365 mμ, tip curvature; □, 365 mμ, base curvature.

for high doses, providing these are given over at least a 4-minute period. As is clear from Table I (see also references *20*, *35*, and *36*), it is possible to get small but distinct negative curvatures in the dose range between first and second positive curvatures, but only providing that the light intensity is high and, correspondingly, the exposures short. At the other extreme, if the light intensity is low, long exposures are necessary if high doses are to be administered, and under these conditions the depression in the response curve disappears; as a result the first and second positive curvatures become indistinguishable. By suitable modifications of duration and intensity, it is easily possible to telescope the first and second positive curvatures together, as the authors' own experiments and more extensive studies of Briggs (*37*) have shown. These re-

sponses are summarized diagrammatically in Fig. 3. Conditions for obtaining the "step-shaped" curve I_1 of Fig. 3 have been carefully studied by Briggs (*37*). It should be added that duBuy and Nuernbergk (*16*) reported a second indifferent range and "third positive" curvatures at still higher doses, but these experiments involved very long exposures, approaching the continuous, to very bright light.

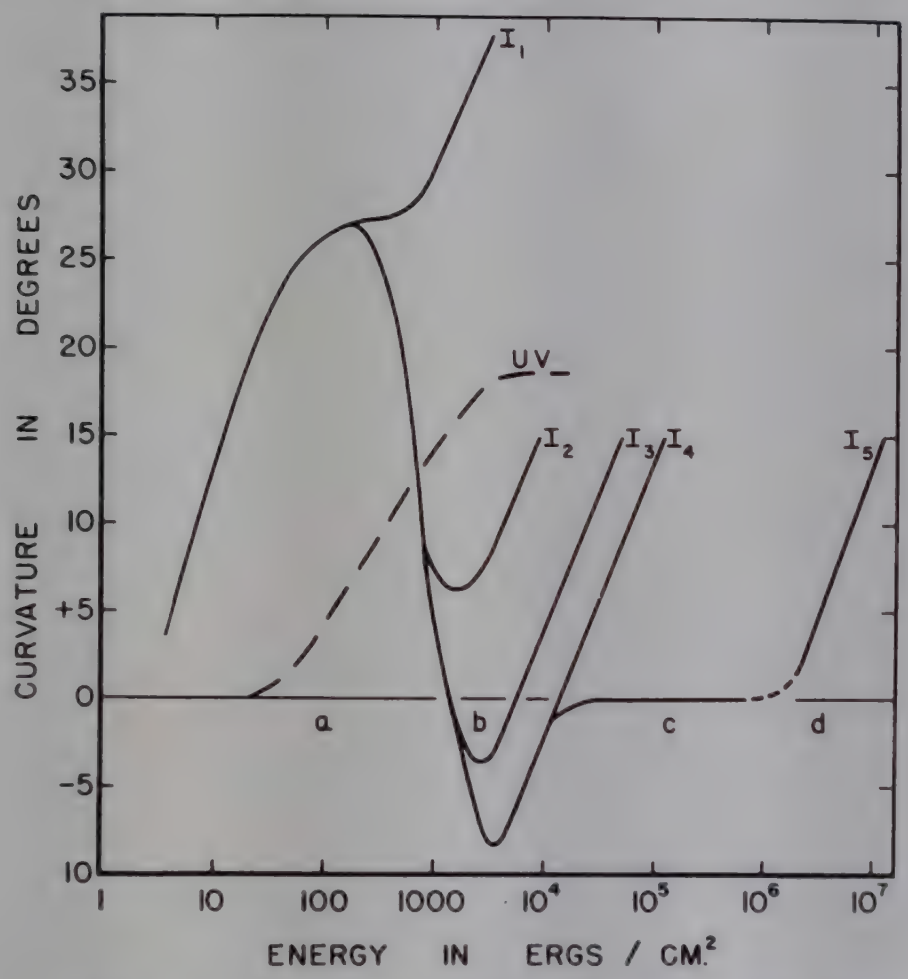


FIG. 3. Diagram of the phototropic responses of the *Avena* coleoptile. Solid lines, blue light (436 mμ); broken line, ultraviolet light (280 mμ). Ordinate, curvature observed 90 minutes after beginning the exposure; abscissa, log dose ($= \log I \times t$). *a*, range of doses giving first positive curvature; *b*, negative curvature; *c*, indifference; and *d*, second positive curvature, observed with very high light intensity I_5. Note that as the light intensity is diminished (I_1, I_3, etc.) ranges *c* and *b* successively disappear, until at very low intensities (exposures approaching continuous) only positive curvatures are observed. Based on data of many workers.

Beside the differences between "tip" and "base" responses mentioned previously, further evidence for a dual mechanism comes from experiments in which particular parts of the coleoptile were exposed. Darwin noted that exposures of the tip alone caused curvatures to develop in the subapical regions. Lange (*38*) found that the apical ½ mm. region is at least 800 times as sensitive to light, in terms of the requirement

for curvature, as any ½ mm. region more than 1.5 mm. down from the apex. Since the apical ½ mm. grows very little and the curvature caused by light reception in this zone develops in more actively growing regions far below, some kind of stimulus transmission is indicated; alternatively, then, the "tip response" could be called a "transmitted response." Historically indeed it was the transmission of the response from the illuminated tip to the unilluminated part below that led to the discovery of the role of auxin in growth and tropisms.

In contrast, prolonged high-intensity visible light or shorter exposure to ultraviolet causes growth changes and curvatures that are not far removed from the site of light reception. The curvatures toward ultraviolet light (wavelength less than 320 mμ) are quite similar to the "base responses" discussed previously, both in their final appearance and in the time course of their development (Fig. 1, *b*). With light of wavelength 280 mμ, large base curvatures are produced free from the complicating factor of simultaneous tip curvatures, and thus more amenable to analysis (see Section VII, B below). The most sensitive zones for the base response are the actively growing regions just below the tip and around the node at the base of the coleoptile. The convenient term "base response" is a little misleading because the response actually occurs all over the coleoptile. However, even the extreme tip can give such responses (*36*, *39*), though the lower regions are more sensitive. The main point is that the base response is localized, in the sense that the curvature takes place at or near the site of light reception.

If the tip is covered during unilateral exposure to white light, curvatures are not observed 90 minutes later unless light doses are given which fall in the range of the second positive curvature, i.e., above 5000 ergs cm.$^{-2}$ given over a period of 4 minutes or more. This suggests that the first positive and negative curvatures are attributable to the tip response, while the second positive curvatures arise largely from the base response. This view is confirmed by the different shapes of the curvatures in the two cases (Fig. 1). With continuous, low-intensity exposures of the whole coleoptile like those of the early experiments discussed above, it is impossible to decide which reaction accounts for the large curvatures observed; probably both mechanisms operate under these conditions (see curve I_1 in Fig. 3).

To produce the first observable or "threshold" curvatures Blaauw found that a definite quantity of energy was required (the *Reizmengengesetz*). Thus, either a 1/1000 second exposure to an intensity of 26,520 meter-candles [26.5 meter-candle-seconds (MCS)] or a 40 hour exposure to 0.00017 meter-candles (26.3 MCS) caused a slight curvature toward the light in about half the *Avena* coleoptiles observed. Blaauw's

work put the study of phototropism on a sound quantitative basis for the first time. Most of his conclusions, however, were drawn from threshold responses, and later workers have found it more accurate to determine actual curvatures. In this way it has since been found, at least in *Avena* (*36*), that the degree of phototropic curvature is determined by the incident light energy ($I \times t$, Reciprocity Law, or Bunsen-Roscoe Law) as long as t is small, just as found by Loeb for the light responses of animals (Section II, above). However, as shown in Table I, for longer exposures the response is mainly determined by t.

B. Types of Phototropism in *Phycomyces* Sporangiophores

The sporangiophores of *Phycomyces* go through four distinct growth stages (*40*) at the end of the third of which the sporangium is fully formed and beginning to blacken. It is only in the ensuing fourth stage that rapid growth and high phototropic sensitivity are found. Accordingly, virtually all of the discussion of phototropism in *Phycomyces* refers to sporangiophores in stage four.

In these organs, as in coleoptiles, the phototropism takes several different forms. The results of Blaauw (*32*) are summarized in Table II.

TABLE II

Responses of *Phycomyces* to White Light[a]

Response	Dose in meter-candle-seconds
Threshold (50% give transient positive curvature)	100–150
Maximum positive curvature	800–1500
Decreasing positive curvature	3000
Some plants show negative curvatures	10^5–2.10^5
Long-lasting negative curvature	2.10^5 and above

[a] Data of Blaauw (*32*).

It will be seen that change of sign in the direction of curvature occurs at a white light dosage approximately equivalent to 4000 ergs/cm.^{2} of blue light.* However, Blaauw noted that this behavior only occurs when high intensities are used; at low light intensity, even continuous exposures yield large positive curvatures. The origin and significance of the negative curvatures observed by Blaauw were subsequently questioned by Castle (*41*) who gave evidence that they were due to temperature effects.

Recently Reichardt and Varju have shown that if *Phycomyces* sporangiophores, which are developing a positive curvature (*42*), are sub-

* Making use of the approximate phototropic equivalence of 1 MCS of white light and 0.04 erg/cm.^{2} of blue light (cf. reference *16*).

jected to a sudden increase in illumination, the curvature undergoes a transient decrease, i.e., they temporarily reverse the sign of their phototropism. While the reversal is not large enough to cause a *net* negative curvature, it does show that curvature away from visible light is possible under some conditions.

In continuous ultraviolet (wavelength less than 310 mμ) actively growing sporangiophores bend vigorously *away* from the source, often reaching 90 degrees of negative curvature in 20 minutes (*43*) (Fig. 4).*

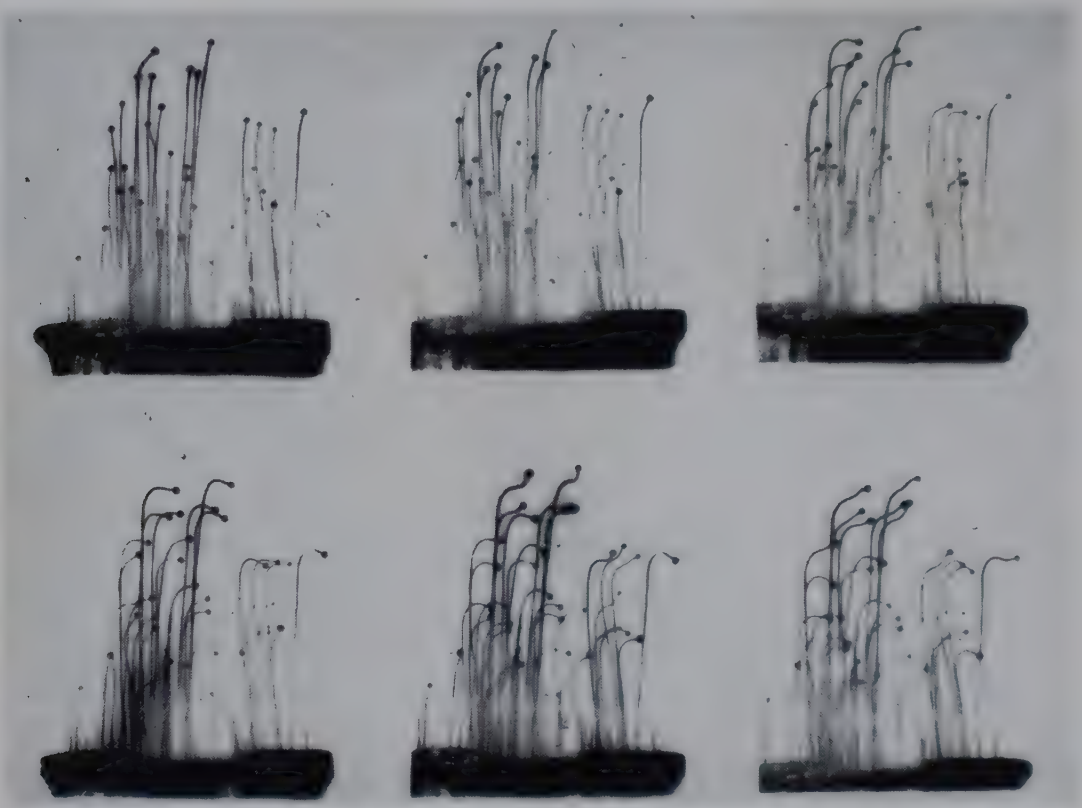


FIG. 4. Negative curvature of *Phycomyces blakesleeanus* induced by continuous ultraviolet (coming from the left). Time after beginning of exposure: left to right (upper row) 5, 15, 25 minutes; (lower row) 35, 45, 55 minutes. (From Curry and Gruen, *43*.)

The growth zone at stage four in *Phycomyces* sporangiophores is limited to a narrow region only 2-5 mm. long below the sporangium. This region must be exposed for any reaction to occur and both positive and negative phototropic curvatures are generated here. Therefore, the response is localized and in this sense more like the base response in *Avena*, though there is some evidence that the curvature may be translocated, at least within this zone (*44*). In terms of the energy requirements and the nature of the dose-effect curves, moreover, some of the responses of *Phycomyces* are more similar to the "tip response" in *Avena*. All in all,

* All of the 5 strains of *P. blakesleeanus* tested (both + and −) and 1 strain of *P. nitens* have given this reaction.

there is an interesting semiquantitative parallel between the behavior of *Phycomyces* and that of *Avena*.

C. The Relation Between Curvature and the Light-Growth Reaction

It was the concept of Blaauw that phototropic curvature depended on the difference between the "light-growth reactions" of the dark and bright sides of a growing organ. These reactions, i.e., changes in growth rate brought about by light, could be positive or negative, simple or complex. They were considered as taking place quite independently on the two sides, and as a function of the light dosage, $I \times t$, on each side.

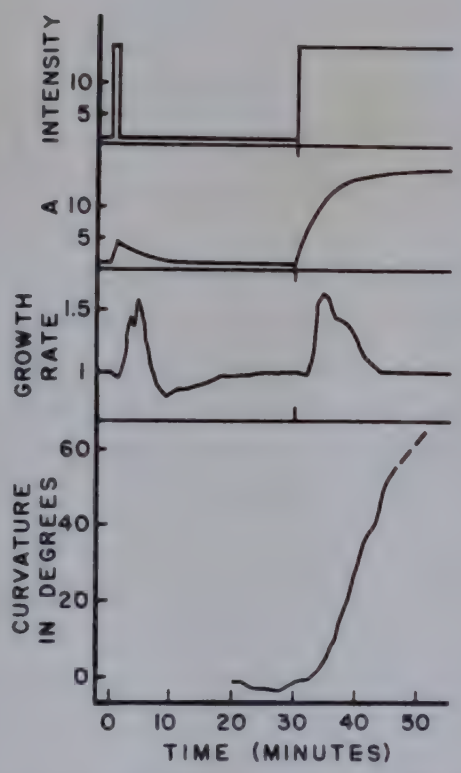


FIG. 5. Diagram of light-growth reaction and phototropism in *Phycomyces*. Top, illumination program; next, the level of adaptation, *A*; next, the growth rate, showing two light-growth reactions, the first after a light pulse, the second after change to continuous high light intensity; bottom, phototropic curvature with continuous high light intensity supplied unilaterally. From Cohen and Delbrück (*44*), and Delbrück and Reichardt (*45*).

In *Phycomyces* short, symmetrical exposures to white light or to ultraviolet cause a temporary acceleration of growth, followed by a slight depression (Fig. 5). If the plants are brought from darkness and subjected to continuous illumination, the growth rate rises for 10 or 15 minutes and then falls back to the normal rate. Castle (*45a*) has recently shown, using a brief light exposure, that the acceleration is

distributed over the whole growth zone, i.e., from about 0.2 to 3 mm. below the sporangium. The growth rate of each part of the growth zone was increased by the same factor, which in one case was 2.43. The light exposure used was 180 ft.-candles white light for 20 seconds, or about 30,000 MCS. A comparable, though still more transient, increase in growth rate occurs when the plants are briefly exposed to ultraviolet (43).

Thus, the negative curvatures which are caused by ultraviolet can be ascribed to an acceleration of growth (positive light-growth reaction) on the front side. With visible light, however, the paradox is that the curvatures are positive although the growth rate is accelerated. To account for this, Blaauw (46), Buder (47), Castle (48), and others have assumed that one-sided light somehow exerts a greater net growth-accelerating effect on the back side of the plant than on the front, and have explained this as due to the lens-like action of the clear cylindrical cell, which produces a bright stripe of high light intensity in the center of the back wall and thus accelerates growth there more than anywhere else. Buder indeed showed that when the plants are immersed in mineral oil, whose refractive index is high enough to prevent formation of the bright stripe, continuous white light now causes a negative curvature.

An interesting parallel case was studied by Dassek (49) in the rhizoids of the liverworts *Marchantia* and *Lunularia*. These clear, unicellular, hair-like outgrowths are negatively phototropic and exhibit a negative light-growth reaction. Since they are so transparent the explanation given for *Phycomyces* was invoked. Furthermore, immersion in liquid paraffin caused the curvature to become positive. An exactly similar situation holds in the germinating mycelia of *Puccinia* and *Uromyces* (50) which also show negative phototropism, a negative light-growth reaction, and reversal of the tropism in paraffin oil. Unfortunately the support to the lens interpretation offered by the experiments in paraffin oil is weakened by Ziegler's finding (51) that a similar reversal of sign of curvature takes place when *Avena* is immersed in oil. The coleoptile, of course, is multicellular and relatively opaque.

The intensity requirements, time courses, and magnitudes of the light-growth reactions of *Phycomyces* match those of the short exposure (transient) curvatures, in accord with Blaauw's theory. The same is true for exposure to ultraviolet. However, in continuous light we encounter another and more serious paradox. The light-growth reaction in symmetrically illuminated sporangiophores comes to an end after about ten minutes, a fact which is ascribed by Delbrück and Reichardt (45) to the onset of "adaptation" (see A in Fig. 5). The level of adaptation, A, is defined as the intensity with which the specimen will fin

self in equilibrium, it is proportional to the light stimulus necessary o evoke a given light-growth reaction.

In darkness this adaptation level is at a minimum (zero); after a rief exposure to light it increases temporarily and then decreases again o this minimum (45). There is good reason to believe that the decrease s exponential (52). As can be seen in the figure, however, its level increases steadily in *continuous* light and correspondingly the light-growth eaction returns to zero. If, however, the illumination is not symmetrical, phototropic curvature begins after about the same reaction time as hat of the light-growth reaction, but *it continues to increase linearly* nd is still increasing when the light-growth reaction has died away Fig. 5). This discrepancy is indeed hard to explain and it indicates hat the relation between light-growth reaction and curvature is very omplex.

In *Avena* the situation is also complex, though perhaps better understood. Table III summarizes the pertinent information about its light-rowth reactions, following symmetrical illumination, and compares them with its phototropism.

The existence of two light-growth mechanisms, differently distributed n the plant, is apparent here. Indeed the existence of a rapid and a low light-growth reaction was made clear as long ago as 1925 by Went 53). With intermediate doses or prolonged low intensity exposures it s probable that both systems operate simultaneously. Analysis of such nixed responses accounts for the wide array of facts and theories concerning the connection between light-growth reactions and curvature.

It is quite clear that the two phenomena are closely related. The ight-growth reactions produced by long exposures or high doses, although they are not proportional to the product $I \times t$, in other respects orrespond well to Blaauw's concept, in the sense that they occur more r less independently throughout the growing regions and that their ffects are additive. In this respect they are closely allied to the *Phycomyces* reactions. These reactions can be most simply interpreted as direct, light-produced changes in growth rate at, or very close to, the ite of light absorption, i.e., localized reactions. The changes are transient nd are soon compensated, as if light temporarily unbalances the normal equence of events in the growth system.

The "tip responses," however, seem to depend on the special properies of the tip and not to occur in the plant as a whole. The growth hanges produced are, in part, spatially removed from the site of light bsorption, i.e., transmitted reactions. They probably result from a nore long-lasting interference by light in the production and distribuion of a growth-controlling factor, generated in the tip and active in

TABLE III

COMPARISON BETWEEN THE GENERAL PROPERTIES OF THE PHOTOTROPISM OF *Avena* AND ITS LIGHT-GROWTH REACTIONS[a]

	Tip Response (Optimum Stimulus: Short Exposure; Low dose)	Base Response (Optimum Stimulus: Long Exposure; High dose)
Properties of the curvature:		
Direction and maximum magnitude	Positive, approx. 30°	Positive, approx. 18°
Shape	Concentrated in tip ("tip" curvature)	Distributed over length ("base" curvature)
Location of sensitivity	Tip	Entire plant, especially growing regions
Time course	Starts slowly (30–40 minutes) near tip, continues to develop for three hours or more, migrates basally	Starts quickly (10–15 minutes) along entire length exposed; reaches complete development in 90 minutes, then regresses
Change with increasing doses	Positive, zero, negative, zero	Increasing positive
Properties of the light-growth reaction:		
Direction and magnitude	Slight depression of growth rate	Sharp depression of growth rate
Location of sensitivity	Tip ("tip" response)	Entire plant, especially subapical ("base" response)
Time course	Starts slowly, prolonged	Starts quickly, reverses, followed by small acceleration
Change with increasing intensity	Depression decreases towards zero (effect vanishes)	Depression increases to maximum

[a] Data of Arisz, Went, van Dillewijn, Haig, and the authors.

lower regions. Asymmetrical interference would lead to asymmetrical distribution of this factor, hence curvature. Additionally, there is some evidence that high-intensity light may remove this interference (cf. decreasing light-growth reaction), possibly accounting for negative curvatures, i.e., more interference on the shaded side than on the illuminated side after a short high-intensity exposure. This aspect of the phototropic responses will now be taken up in detail.

V. The Role of Auxin in Phototropism of Plants

In contrast to the Blaauw theory, the Cholodny-Went theory (*54, 55*) postulated that any tropistic curvature, whether caused by light or gravity, is due to the lateral displacement of the growth-controlling substance (now termed auxin). In the normal geotropic curvature of shoots auxin would move towards the lower side; in positive phototropism auxin would move to the dark side. This movement of auxin necessarily would cause light-growth reactions on both sides, a deceleration on the bright side and an acceleration on the dark side.

TABLE IV

DISTRIBUTION OF AUXIN ON THE TWO SIDES OF THE *Avena* COLEOPTILE AS A RESULT OF LIGHT TREATMENTS CAUSING FIRST POSITIVE, INDIFFERENT, NEGATIVE, AND SECOND POSITIVE CURVATURES

Amount of light (meter-candle-seconds)[a]	Phototropic curvature degrees	Auxin distribution in per cent		Author
		Light side	Dark side	
0	0	49.9	50.1	Asana (*56*)
20	approx. +10	41.0	59.0	Went (*55*)
100	++	26.0	74.0	Went (*55*)
1,000	+48	32.0	68.0	Went (*55*)
1,500	+50	17.0	83.0	Wilden (*57*)
10,000	approx. 0	49.0	51.0	Went (*55*)
11,400	−15	62.0	38.0	Wilden (*57*)
?	−20	58.0	42.0	Asana (*56*)
150,000	+20	36.0	64.0	Wilden (*57*)

[a] One meter-candle-second of white light is phototropically equivalent to about 0.04 erg/cm.^{2} of blue light.

That such an auxin differential is indeed set up was proven by Went (*55*) for the first positive curvature of *Avena* coleoptiles and the proof was extended by Asana (*56*) and Wilden (*57*) to negative and to second positive curvatures. The data are summarized in Table IV. Recently Briggs *et al.* (*58*) have obtained similar results on corn coleoptiles, using light dosages corresponding to first and second positive curvatures. Experiments with some dicotyledonous seedlings at low light intensities

are summarized in Table V. They bear out the general conclusion of the work with *Avena* coleoptiles that curvature towards light is associated with a decreased auxin level on the lighted side and an increased auxin level on the dark side.

In these experiments the auxin was usually bio-assayed by placing the tip of the coleoptile or other seedling in contact with a block of agar, which was so divided that the yields of auxin from the bright side and from the shaded side were received in separate blocks. Each agar block was then applied to one side of a standard decapitated Avena coleoptile and the curvature resulting after 110 minutes measured. It is of interest, however, that when the auxin was obtained by slitting the seedling lengthwise and extracting the two halves with solvent (with subsequent bio-assay on Avena coleoptiles) the same auxin differential could be established (*20*, 59) (see Table V).

If the auxin differential, in a positive curvature, genuinely comprises both a decrease on the light side *and* an increase on the dark side, it would follow that the auxin must have moved laterally, under the influence of light, from front to back. Recently, however, it has been suggested a number of times that the effect of light in causing curvature is exerted through the destruction of auxin on the light side. This would of course mean only a decrease of auxin, and therefore of growth, on the light side, with no corresponding increase on the dark side. In Blaauw's terms it would mean a negative light-growth reaction on the light side only. For a biochemical interpretation it is a crucial question whether the difference in auxin content on the two sides is due to destruction on the lighted side or not.

At this point it is therefore worthwhile to review the evidence which specifically indicates lateral movement of auxin, as opposed to destruction on one side. The first experiment to give such evidence was that of Boysen-Jensen and Nielsen (*60*) and Boysen-Jensen (*61*) in which a coleoptile was split at the tip by the insertion of a thin sliver of mica. When the plant was illuminated perpendicular to the plane of the mica, the curvature was greatly reduced. Controls illuminated parallel to the plane of the mica curved normally, thus eliminating the effect of slitting injury. These experiments were carried out using prolonged light exposures, and must be repeated using about 100 ergs cm.$^{-2}$ to bring the curvature down into the first positive range. An opposite indication comes from simultaneous measurements of growth rate and development of curvature, from which duBuy and Nuernbergk (*62*) calculated that the slowing down of growth on the lighted side was almost complete, and much greater than the acceleration on the dark side.

The work based on auxin determination was at first not quite con-

TABLE V

REDISTRIBUTION OF AUXIN CAUSED BY UNILATERAL ILLUMINATION OF DICOTYLEDONS[a]

Plant used	Material and method	Illumination time (hours)	Plants unilaterally illuminated			Plants in dark		
			Light side	Dark side	Total	Side A	Side B	Total
			(Curvature in degrees)			(Curvature in degrees)		
Raphanus sativus	Hypocotyl tips; 3 hours diffusate	2	1 ± 1	4.4 ± 1.5	5.3	5.1 ± 0.8	5.0 ± 1.5	10.4
		1	0.8 ± 1	5.8 ± 1	6.6			
Raphanus sativus	6 mm. cylinders of hypocotyl, with auxin applied; 3 hours diffusate	3	6.6[b]	11.0[b]	17.6	10.0[c]	9.9[c]	19.9
			(Auxin units/100 gm. dry weight)					
Phaseolus multiflorus	Light-grown stems; $CHCl_3$ extraction	2^1_2	2.9[d]	5.3[d]	8.2[d]			
	Dark-grown stems; $CHCl_3$ extraction	4	2.5[b]	3.6[b]	6.1			
		4	17.4[d,e]	29.8[d,e]				

[a] Data of van Overbeek for *Raphanus* (*17*); Boysen-Jensen for *Phaseolus* (*59*).

[b] Average of 8 experiments.

[c] Average of 2 experiments.

[d] Average of 4 experiments.

[e] Another variety, of higher auxin content.

vincing; Went's data, for instance (*55*) showed a much greater decrease in auxin on the lighted side than increase on the dark side. This agrees with the growth measurements (*62*) just mentioned. Van Overbeek's data with *Raphanus* tips (*17*) show the same thing, provided the auxin is being produced in the tissue; they do not show it for auxin externally applied to tissue cylinders (Table V). Oppenoorth (*20*) using ether extraction and *Avena* coleoptiles, found less auxin on the lighted side than in dark controls, but in some experiments the increase on the dark side began only after two hours, while in others it never did occur, both sides showing less auxin than in the unilluminated controls. At light dosages causing negative curvatures he found a greater increase on the light than on the dark side, but both sides showed *more* auxin than before illumination. Thus in positive curvatures there appears to be some over-all destruction by light, or in negative curvatures some over-all promotion of auxin synthesis. Söding, in his book, concludes: "These two factors make it difficult to conclude with complete certainty, from *relative* figures of the auxin content of the dark and light sides, that there is lateral movement of auxin, since all the differences can theoretically be equally well ascribed to a reduction or an increase of the auxin content by light" (*63*). However, Briggs *et al.* (*58*) repeated and extended experiments of the type done by Went, but using corn (*Zea mays*) coleoptiles bisected at the base. When placed on agar in the dark or in all-round light the auxin yield from the two sides was satisfactorily the same (Fig. 6) and when illuminated from one side, that side yielded less auxin than the other, as expected. But if the bisection went all the way up to the tip (perpendicular to the direction of illumination), so that lateral movement could not occur, then the auxin yield from the two sides was the same. This experiment seems quite convincing and one must conclude that the effect of low intensity light is exerted, not on the auxin itself, but on the system which produces and/or transports it.

A similar conclusion follows from an entirely different consideration, namely from the quantum yield of phototropism. Bünning (*64*) came to the conclusion that the energy input for tip responses was too low by a factor of 10^4 (assuming a quantum yield of unity) to account for sufficient auxin destruction. An estimate along similar lines follows.

For highly sensitive coleoptiles a dose of 1 erg/cm.^{2} of 440 mμ light applied on 1 mm.^{2} of the apical surface will suffice for a 5° curvature. This dose represents approximately 2×10^{11} quanta/cm^2 $\times 10^{-2}$ cm.^{2} $= 2 \times 10^9$ quanta. Making the generous assumption that 10% of these quanta are involved in a one-for-one inactivation of auxin molecules on the front side of the coleoptile, one would expect a difference of 2×10^8

auxin molecules in the two sides (assuming none are inactivated in the back side). The one-sided application of a 10 mm.^{3} agar block containing 3-indoleacetic acid (IAA) at a concentration of 10 μg./liter will produce a 5° curvature in the standard *Avena* test. Since this concentration is $10 \times 10^{-6}/175 = 6 \times 10^{-8}$ molar, and the block volume is approx. 10 μl., the auxin differential required is $6 \times 10^{-8} \times 10^{-5} \times 6 \times 10^{23} = 4 \times 10^{11}$ molecules. However, since only 15% of the applied auxin goes in during the 90 minutes (65), this becomes a little less than 10^{11} molecules, or

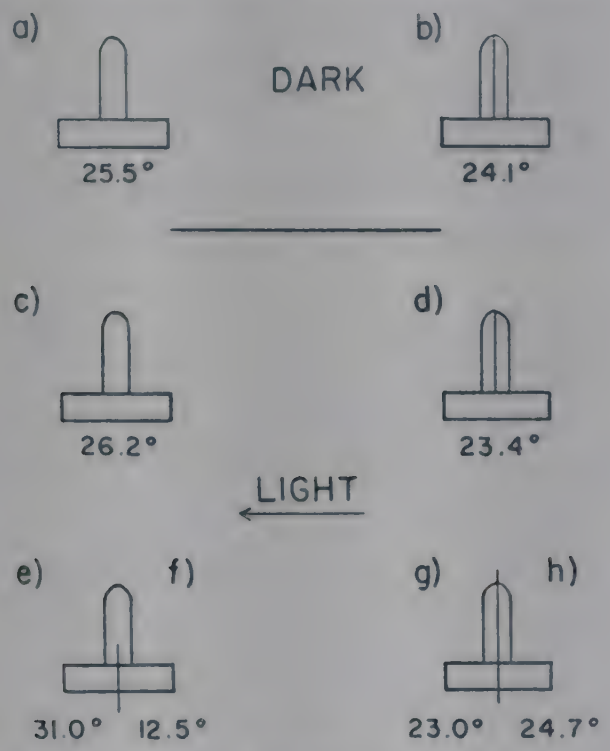


FIG. 6. Diffusion of auxin into agar in three hours from corn coleoptile tips: (*a*) three intact tips, dark; (*b*) three totally split tips, dark; (*c*) three intact tips, light; (*d*) three totally split tips, light; (*e*) six partially split tips, dark side; (*f*) six partially split tips, light side; (*g*) six totally split tips, dark side; (*h*) six totally split tips, light side. Auxin given in degrees curvature of test *Avena* coleoptiles, on which 25 μg./liter of IAA gave 9.5°. From Briggs *et al.*, (58.)

3×10^{-11} gm.; this calculation agrees well with that of Reisener (66) who used smaller agar blocks of which 40% of the IAA goes into the plant, and obtained a value of 2×10^{-11} gm. for 10° curvature. This corresponds to at least 400 times as many molecules as can be accounted for on a direct photoinactivation basis, unless quantum yields greater than unity are involved.

In contrast to this indicated quantum yield of 400, determinations of the quantum yield for the ultraviolet photooxidation of IAA in aqueous solutions or at physiological concentrations in agar blocks have given values close to 0.2. Doses of the order of 10^5 to 10^6 ergs/cm.^{2} are required for appreciable inactivation. For the riboflavin sensitized photo-

oxidation of IAA, Galston (*66a*) reported a quantum yield of approximately 0.7. Burkholder and Johnston (*67*) found significant amounts of light-destruction of growth substances in intact and excised tips of *Avena,* and of *Zea mays* and *Nicotiana* growth substance diffused into agar, but only by the application of relatively tremendous doses (10^6 to 10^8 ergs/cm.^{2}). In similar experiments the authors have found that "natural" auxin from *Avena* tips is inactivated by ultraviolet light, but with efficiencies no greater than the inactivation of IAA. Recently Reisener (*66*), using C^{14}-labeled IAA introduced into the coleoptile by diffusion, could find no light-destruction with a white light dosage about equal to 10,000 ergs/cm.^{2} of blue light. There was also no destruction of IAA in solution with this dosage. All these facts argue strongly against the possibility that significant amounts of growth substance could be inactivated by light in doses of a few ergs per square centimeter.

In connection with the postulated effect of light on auxin distribution, an important generalization must be made: Wherever auxin is produced by the plant, polar transport away from the production site also occurs. If production were more rapid than transport, auxin would accumulate locally and tumors and swellings would result. This seems to happen only when the auxin is produced by agencies other than those normally in the plant, i.e., by microorganisms. Accumulation does not occur (except in fruits) when the auxin production is by the plant's own normal tissues. Correspondingly, in the absence of auxin production, polar transport disappears; thus it has been found that in *Pinus palustris,* which does not elongate for the first seven years of life, auxin is not destroyed, but *both* the auxin production *and* the polar transport system are absent (*68*). It seems, then, that production and transport are interlinked, and the molecules of auxin are transported away from the site of formation in a process which is integral with the formation reaction. Under the influence of light, it must be this system which is modified in such a way that the molecules of auxin, as they are formed, are transported abnormally. This might mean that under the influence of light the "polarity" in the tissue changes from longitudinal to lateral, or it might only mean that light paralyzes, or retards, the transport system; as a result the produced auxin would then accumulate and some of the excess would find its way to the dark side and be transported downwards there. Alternatively, light might paralyze or retard the system which forms the auxin from a precursor. As a result, excess precursor would accumulate and some of this would be converted to auxin on the dark side. The fact that the total auxin yield does not change when the tips are illuminated and bisected, however (*58*), does not

favor either of the latter explanations. Whichever mechanism operates, it must be some aspect of the auxin-*producing* system which is light-sensitive.*

This conclusion is supported (in a rather negative way) by some recent experiments using artificially applied auxin (*66*, *69*, *70*). In these, isotopically labeled indoleacetic acid was applied to the tip of a coleoptile and then after unilateral illumination the distribution of isotope was followed. In Bünning's case a thin section from the extreme tip was removed and the auxin then applied to the cut surface in agar, which, as Brauner (*71*) points out, may have removed just the tissue able to carry out auxin redistribution. However, Reisener (*67a*) repeated the experiment with intact coleoptiles to which a blocklet of IAA in agar was applied. The results of the three workers were the same, namely that the C^{14} content of the bright and dark sides was identical within the limits of counting error. Although the authors cited have drawn different conclusions, the only reasonable one appears to be that *preformed* indoleacetic acid is not acted on by the light, and therefore that the action occurs at the site of auxin production.

For the sake of completeness, it should be mentioned here that in recent years it has become clear that a second powerful growth-controlling factor, namely gibberellin, operates in plants. However, since the characteristic of gibberellin-induced growth is that it takes place *par excellence* in the light (*72*), it is highly improbable that gibberellin plays any major part in phototropism.

VI. Nature of the Photoreceptors

A. Photoreceptors for Phototaxis

From the biochemical viewpoint, one of the major problems in the study of a light reaction is to identify the photoreceptor, and this in turn can best be approached by optical methods through determination of the action spectrum. But action spectra cannot be used to determine the absorption spectrum of the light absorber unless they are both precise and detailed. For this reason many of the early observations as to the spectral regions effective for phototaxis give little basis for reliable deductions about the photoreceptor.

The first organisms to be studied carefully for their spectral sensitivity were the purple bacteria, especially *Rhodospirillum rubrum*. As

* This argument probably does not hold for the base response, since it is possible to apply auxin *after* illumination of decapitated *Avena* coleoptiles and obtain curvature (see Section VII, B). Here the light must act upon a transport system which remains laterally polarized for some time afterwards.

long ago as 1882–1888 Engelmann observed that these anaerobic organisms, if they swim into a region of lower light intensity, undergo a "shock reaction" which changes their direction of movement. Since this occurs whenever they cross the boundary into a darker zone, the result is that the organisms accumulate in the lighted area ("phobo-phototaxis"). If now they are illuminated with a small spectrum the bacteria will move towards, and collect in, the following specific regions of the spectrum: the infrared from 800 to 900 mμ, a band in the yellow near 590 mμ, and bands in the green later specified by Buder as 530, 490 and 470 mμ. The absorption spectrum of a suspension of the cells shows bands in just the same places, with an additional one at 550 mμ. Those at 880 and 590 mμ have since been identified as belonging to the green pigment bacteriochlorophyll, the others to a series of carotenoids (*73*, *74*). The deduction is, then, that all of these pigments act as photoreceptors for phototaxis. A complication is that the extracted and purified pigments do not show peaks at quite the same wavelengths as in the living cell, due to their combination with protein *in vivo*. Nevertheless the spectral bands of the pigments could be fairly well identified with those in the action spectrum.

The significance of the chlorophyll and carotenoids as photoreceptors lies in the fact that the purple bacteria, as Engelmann believed and as was proved almost fifty years later by van Niel (*75*) are photosynthetic, and both the chlorophyll and the carotenoids (with the apparent exception of spirilloxanthin, the one which absorbs at 550 mμ) can absorb light active in photosynthesis. The action spectrum for photosynthesis of *Rhodospirillum rubrum*, indeed, shows peaks at about 880, 590, 525, 490, and 460 mμ (*76*) which agree perfectly with the figures for phototaxis. Also the high light intensity causing decreased phototactic sensitivity coincides with that causing saturation of the photosynthetic system (*77*). Thus the phototactic stimulus is considered to result from a sudden decrease in the photosynthetic rate (*74*, *78*).

Engelmann's less extensive experiments under anaerobic conditions with the ciliate *Paramecium bursaria*, which contains endozootic cells of the green alga *Chlorella*, showed a similar behavior, but in this case the cells collected in the region of maximum absorption of chlorophylls a and b, namely around 650–700 mμ. Since these chlorophylls are the main photosynthetic pigments of the green algae, the observations again indicate that the stimulus for phototaxis is a change in the rate of photosynthesis. Indeed Links (*79*) has generalized this conclusion to apply also to chemotaxis and osmotaxis: "The common factor in the chain of processes . . . which give rise to the characteristic shock-reaction is the sudden lowering of the energy consumption in the motility apparatus of

the reacting protist." Approximate measurements with the blue-green alga *Oscillatoria,* which, however, has a gliding motion and is not propelled by flagella, also indicate that the light absorbed by chlorophyll is phototactically active (*80*).

A different type of phototaxis is that shown by motile green algae such as *Euglena* and *Eudorina,* or the zoospores of algae or ferns. These organisms do not merely collect in a light spot, but actually swim in the direction of the light. As described in Section II, such directional phototaxis (called by some authors "topo-phototaxis") can be positive or negative; the positive movement commonly takes place at much lower light intensities than the negative; both are more light-sensitive than the "shock response" of *Rhodospirillum.* Indeed Manten (*74*) concludes that the phototactic threshold for *Rhodospirillum* lies at about 10^4 times higher light intensity than the threshold for the green alga *Eudorina.* Positive phototaxis of *Chlamydomonas* occurs equally well in the absence of CO_2 (*80a*) and is thus not directly related to photosynthesis.

According to earlier measurements made mostly by Mast (*81, 82*) the peak of sensitivity for positive phototaxis of green algae lies always in the blue or blue-green; 524 mμ for *Pandorina* and *Eudorina,* 503 mμ for *Chlamydomonas,* and 494 mμ for *Volvox* — all three in the Volvocales; Mast also found 473–483 mμ for five species of *Euglena.* However, Luntz (*83*) found the peak for *Eudorina elegans* and *Volvox minor* to be at 492 mμ, and Laurens and Hooker (*84*) using a method based on exposure length rather than light intensity, found the same wavelength optimal for *Volvox globator.* Oltmanns (*85*) in a careful study found that *Euglena* shows three peaks, the main one at 480–495 mμ, and secondary peaks at 428 and 405 mμ.

Recent studies in general have agreed well with the above. Bünning and Schneiderhöhn (*86*) found *Euglena* to show four peaks, at about 425, 450, 475, and 495 mμ, two of which agree rather well with Oltmanns' data. However, Halldal very recently (*3*) has found a single peak at 493 mμ for five of the Volvocales with a shoulder at about 435 mμ. Gametes of two *Ulva* species gave a two-peaked or flat-topped curve with sensitivity almost equal from 440 to 490 mμ, while three members of the Dinophyceae each gave a single rather sharp peak, which for *Peridinium* and *Gonyaulax* lay at 475 mμ, and for *Prorocentrum* (an unarmored form) at the surprising wavelength of 570 mμ. Recently *Chlamydomonas* has been found to respond like *Ulva,* showing little change in sensitivity between 465 and 500 mμ; the sensitivity falls off sharply on either side of this range, and reaches zero at 440 and 520 mμ (*80a*). Halldal's action spectra are reproduced in Fig. 7.

Data for negative phototaxis are few; in Halldal's Volvocales the

action spectrum was the same for the negative as for the positive movement, but Bünning and Schneiderhöhn's *Euglena* (*86*) was found to give an entirely different response with the greatest sensitivity at 415 mμ and three small subsidiary peaks towards longer wavelengths. However, the methods were not identical, since in positive phototaxis Bünning and Schneiderhöhn measured the intensity for threshold response,

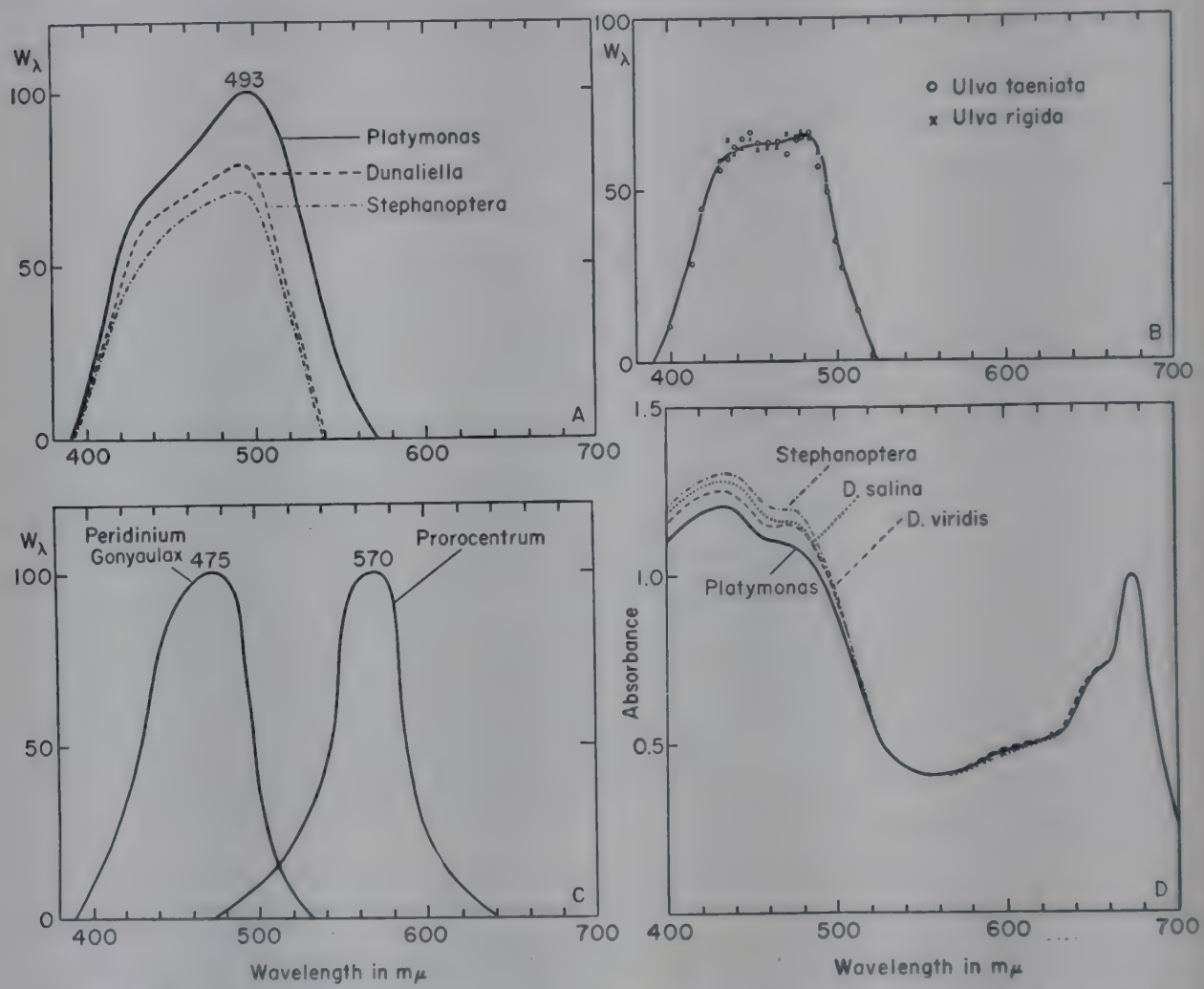


FIG. 7. Phototaxis as a function of wavelength. A, action spectra of phototaxis in three species of *Dunaliella* and two other members of the Volvocales; B, action spectrum of phototaxis in *Ulva* gametes; C, action spectra of phototaxis in three Dinophyceae; D, absorption spectra of living cells of Volvocales. From Halldal (*3*).

while in negative phototaxis they measured the lowest intensity needed to cause reversal. Although the action spectra are not the same, they do fall in the same spectral region, both positive and negative responses reaching zero at 550 mμ. In view of Mast's early observation in 1927 (*87*) that the intensity at which phototaxis reverses can be changed in *Volvox* by adaptation, it seems natural to believe that both directions of movement are activated by the same photoreceptor, and that the change of sign is directly comparable to the change of sign in the effect on

respiration, nerve action, etc., of various drugs and poisons, which stimulate in low concentrations and depress in high.

Lastly there must be mentioned Luntz' study (83) of the nonphotosynthetic *Chilomonas*, which is about 1000 times less sensitive to visible light than the photosynthetic organisms studied, but had a peak of sensitivity in the ultraviolet at 366 mμ (or lower).

The question now is, to what photoreceptors do these action spectra point? It is clear, first of all, that the directional phototaxis of green algae is not directly mediated by the photosynthetic system; in not a single case is the red end of the spectrum photokinetically active. It is virtually certain also that the photoreceptors are different in different plants, though they may fall into two or three groups. The spectra show a general resemblance, both in shape and location, to those of carotenoids, but the similarities are not sharp enough to justify identification with any specific substances. Halldal (3) found that the peak of carotenoid absorption in the spectrum of living Volvocales was at about 440 mμ (see Fig. 7) while the phototactic peak at 493 mμ could correspond only to a minor shoulder on the absorption spectrum. Indeed in *Prorocentrum* the phototactic peak actually falls in a trough of the absorption spectrum. He concludes that "none of the major pigments present in the algae is the photoreceptor." None of the water-soluble pigments, either, showed absorption spectra corresponding to the measured action spectra. It has been suggested that the absorption spectrum of an active carotenoid might well be modified by combination with a protein. The difficulty is that if this is invoked to explain the "flat-topped" action spectra then some other explanation has to be used for the single sharp peaks. It seems necessary to conclude that the photoreceptor is a pigment *present only in traces*, probably but not necessarily a carotenoid, and subject to one or more modifications in different species.

There is another possible explanation of the action spectra, namely that they are actually *difference spectra*, representing the difference between the light absorption of the photoreceptor proper and that of a screening pigment. A number of workers, especially Mast (87) have explained the phototactic stimulus as due to shading of a photoreceptor spot by a screening pigment; the organism moves so as to minimize the shading. (In negative phototaxis it would maximize the shading.) As a rule the screening agent has been thought to be the stigma, an orange-red spot not far from the base of the flagellum; the anatomy of the stigma in *Euglena* has recently been described in detail (87*a*). The stigma contains the carotenoid astaxanthin (88). This pigment has a single absorption peak falling at 470–492 mμ, depending upon the solvent. However, it must be noted that the Dinophyceae, which are photo-

kinetically sensitive, contain no visible stigma, and also that the stigma may be located in quite different positions (relative to the flagellar base) in different algal groups. A mutant of *Chlamydomonas* without stigma was still phototactic, though less rapidly and less regularly so than the wild type, and Hartshorne (*88a*) concluded that "the perception of light in *Chlamydomonas* is a property not restricted to the eyespot." For these reasons the connection of the stigma with phototactic movement is very uncertain (cf. Halldal, *3*). Gössel's finding (*88b*) that for negative phototaxis of chlorophyll-free *Euglena* the action spectrum is the same in strains with and without a stigma shows indeed that for the negative response the stigma is not necessary. Regardless of whether or not the screening function is performed by the stigma, some kind of screening is a likely mechanism for phototaxis. In this case it stands to reason that if the photoreceptor and the screening pigment absorb comparable fractions of the light, the action spectrum will not represent the absorption spectrum of either. (This point is taken up further in Section VI, C, 2.) In general, therefore, the possibility cannot be overlooked that the action spectrum of phototaxis does not represent a simple absorption spectrum but might be a complex of two, perhaps *both* belonging to carotenoids.

The light-directed movements of chloroplasts in the leaves of mosses and flowering plants have something in common with phototaxis. In darkness the chloroplasts tend to be randomly distributed (apostrophe), in weak light they concentrate on the upper surface (epistrophe) and in intense light they withdraw to the side walls, or in the case of the alga *Mougeotia* turn into the profile position, (both considered as parastrophe). These movements, which have been known since the work of Böhm in 1856, are quite slow but very temperature sensitive; for example in *Lemna trisulca* the maximal profile position required 70 minutes at 10° and only 5 minutes at 30° (*89*). The effects of wavelength of light on these movements have been studied a number of times, but have given contradictory results in the hands of different workers and with different plant material. The most recent study, by Zurzycka, (*90*) leads to the conclusion that both red and blue lights are effective but in different ways; epistrophe occurs only in blue light while parastrophe (the profile position) is caused by both blue and red. It is concluded that the movement into or out of the profile position probably depends upon photosynthesis, but the evidence is quite slender. In the first place, NH_2OH, which inhibits photosynthesis without affecting respiration rate, stopped entirely the movements which started from the profile arrangement (*91*). Secondly, there is a parallelism between the light intensities causing the movement and those active in photosyn-

thesis; at that intensity where the linear relation between intensity and photosynthetic rate begins to fall off, the chloroplasts begin to move into the profile position, while at the intensity which saturates photosynthesis the maximum profile position is reached (92). This was true both in *Lemna* and in *Mougeotia,* i.e., a flowering plant and an alga. Further support is derived from the observations of Babushkin (93) on tobacco leaves, in which also the profile position appears to be adopted in light intensities causing saturation of photosynthesis; both movement and photosynthesis are inhibited by phenylurethane. On the other hand, strong evidence that the movement cannot be caused simply by photosynthesis is provided by Haupt's recent observation (*93a*) with Mougeotia that far infrared light of 733 mμ reverses the orientation of chloroplasts caused by red light. The far infrared alone has no effect, so that its action is simply to reverse that of the red. Thus the photoreceptor for chloroplast movement may be the same as that for the far infrared reversible reactions in higher plants, which control stem elongation, photoperiodism, and the germination of light-sensitive seeds. CO_2 participates in the reaction (*93b*).

What the significance of the movement caused only by blue light may be, can only be guessed at. The mechanism of the movements is also obscure, since the plastids are not themselves motile and are evidently carried passively, though directionally, by the circulating cytoplasm. Blue light does change the viscosity of cytoplasm, with a maximum effectiveness between 460 and 490 mμ (*94*) and the streaming rate is highly temperature-sensitive, but the connection of these facts with directed chloroplast movement is obscure.

B. Photoreceptor for the "Tip Response" of *Avena*

The evidence in Section V indicates that the tip response of the *Avena* coleoptile, and perhaps the corresponding reaction in many other plants, is due to a modification in the system producing and transporting auxin, but gives no indication as to what this system is. The auxin of most plants is 3-indoleacetic acid (IAA), which has no absorption in the visible spectrum, yet phototropic curvature occurs in visible light. From the earliest studies (32, 95) it has been generally agreed that, as with the animals discussed in Section II, blue light is most effective in phototropism, red light is least effective. It follows that a yellow pigment must exist which, upon absorption of light, can interact with the colorless IAA system to cause auxin redistribution. Numerous comparisons have therefore been made of the relative phototropic effectiveness of different wavelengths of light (i.e., action spectra) in order to indicate the nature of the primary photoreceptor.

Action spectrum determinations in a variety of higher plants have shown that the region of maximum phototropic sensitivity lies between 420 and 470 mμ. The spectrum falls off much more rapidly toward longer wavelengths than it does toward the violet. The lack of sensitivity at wavelengths above 550 mμ extends to mono- and dicotyledons, liverworts, and several fungi (see reference *16*). Near ultraviolet has generally proved to be less than half as effective as blue, as shown by Blaauw (32) and Sonne (*96*) for *Avena,* and Castle (*97*) for *Phycomyces.* In almost all of the more recent precise determinations two peaks have appeared in the blue (e.g., Johnston (*98*), and Galston and

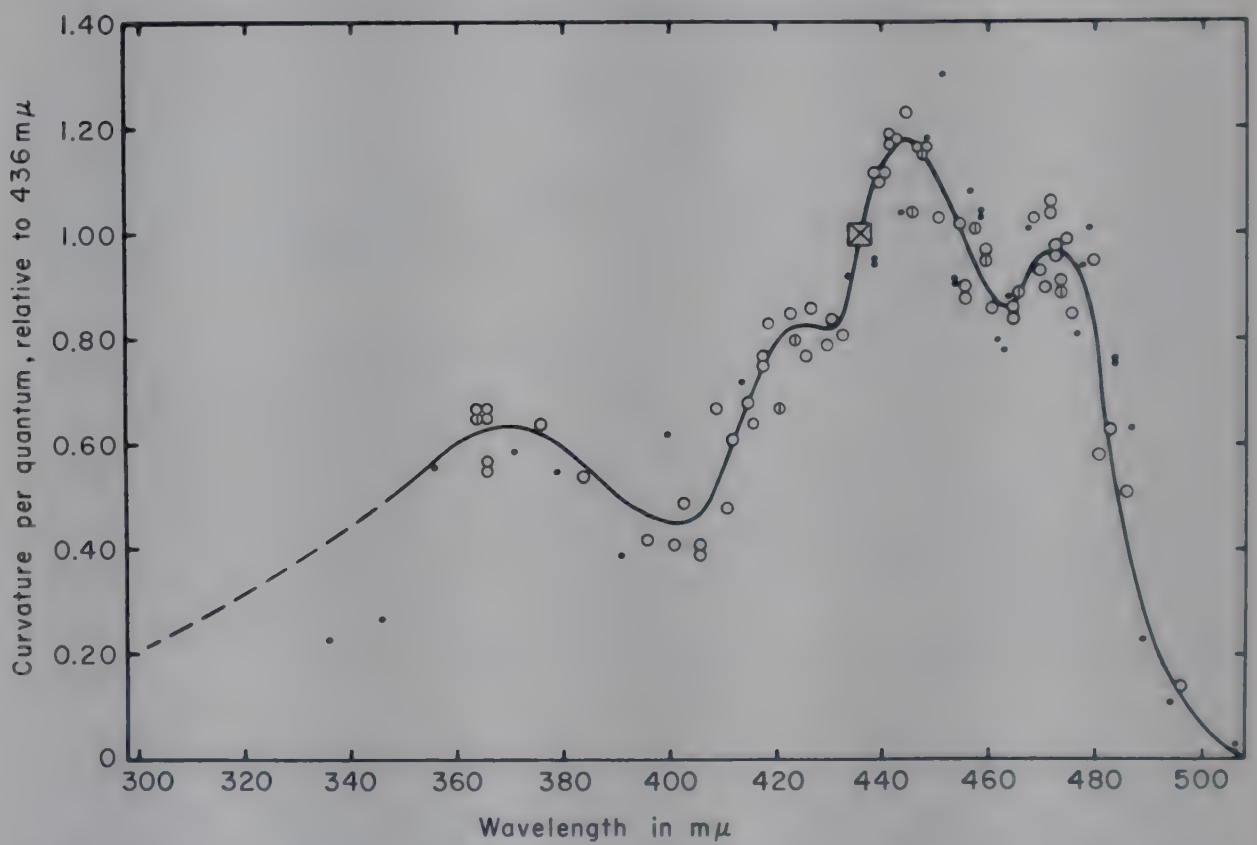


FIG. 8. Action spectrum for the tip response of *Avena* coleoptiles, deduced from the integrated results of three large series of experiments. Large square indicates 436 mμ reference point. Average rows at λ: ●, 1 row; ○, 4 rows; ⊕, 6 rows; ○○, 8 rows. From Curry (*34.*)

Baker (*99*), with *Avena*). In our own laboratory a detailed study of the tip response in *Avena* has given the action spectrum shown in Fig. 8. The existence of two main peaks is confirmed. In addition, there is a definite shoulder below the principal maximum and a small peak in the near ultraviolet. Shropshire and Withrow (*100*) have obtained a similar result with a slightly different method.

In the visible region this action spectrum bears a striking resemblance to the absorption spectrum of a carotenoid pigment (Fig. 9, A). Bachmann and Bergann (*101*) first suggested the possibility of a chromolipid

for the photoreceptor, and their idea was extended, specifically to a carotenoid, by Voerkel (*102*). The two peaks in the action spectrum supported the idea of a carotenoid, and Wald and duBuy (*103*) found carotenoid pigments in the light sensitive zones of *Avena*. In hexane

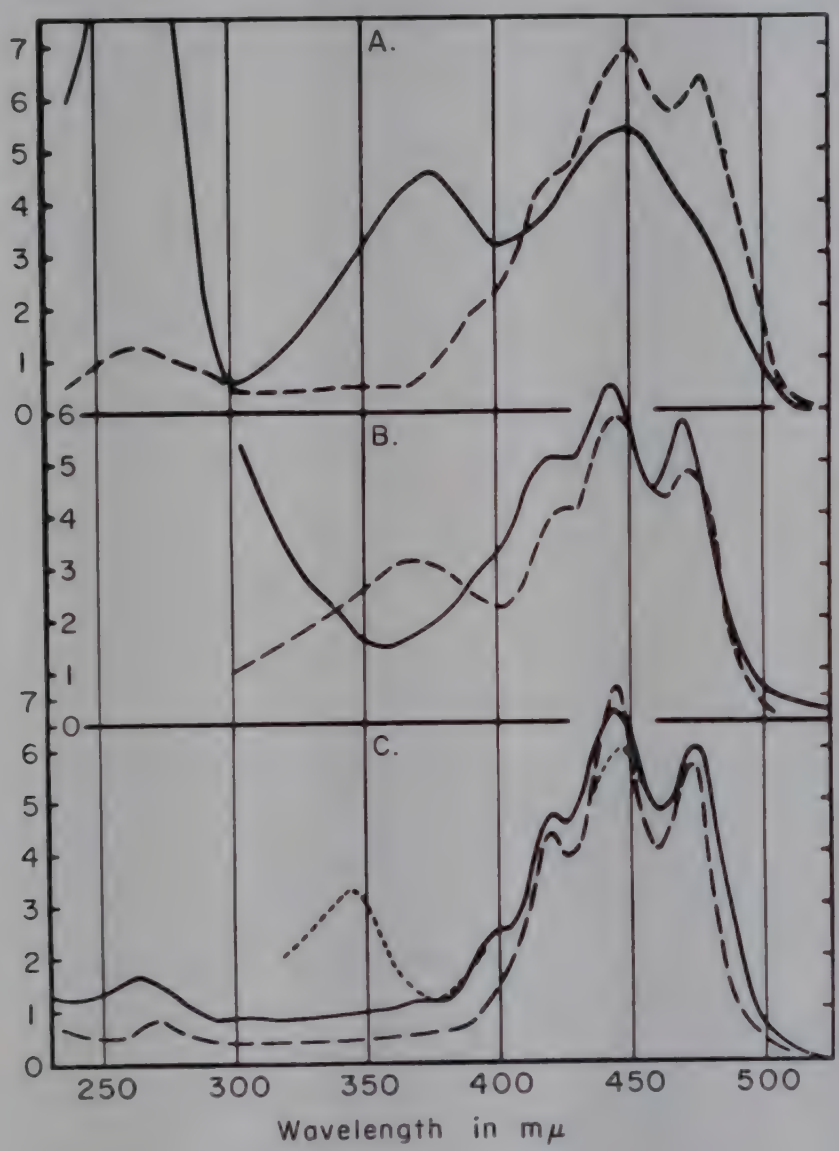


FIG. 9. Comparison of action spectrum and absorption spectra. (All ordinates are arbitrary.) A. Solid line: absorption spectrum of riboflavin. At 265 mμ the riboflavin curve rises to 13.4 on this relative scale. Broken line: absorption spectrum of β-carotene in hexane. B. Solid line: absorption spectrum of a direct hexane extract of 500 coleoptile tips. Broken line: action spectrum for tip response in *Avena* as in Fig. 8. C. Solid line: absorption spectrum of α-carotene in hexane. Broken line: lutein in hexane. Dashed line: 9,9′-mono-*cis*-β-carotene in hexane (*104*). Only the *cis*-peak is shown, together with a few points near the highest peak in the visible as a reference.

extracts of lyophilized coleoptile tips, freed from all pieces of primary leaf, we have observed the absorption spectrum shown in Fig. 9, B. At least three carotenoid fractions are present in this extract, as shown by chromatographic separation, but in any case the similarity between the

absorption and action curves is outstanding. The features important for comparison are listed in Table VI.

It will be noted that the fit is excellent except in the near ultraviolet. There are, however, several carotenoids, present in a wide variety of plant tissues though not yet demonstrated in *Avena*, having absorption peaks in the near ultraviolet. These are *cis* isomers of the more common all-*trans*-carotenoids; for example, the *cis*-peak in the near ultraviolet is shown in Fig. 9, C for one of these stereoisomers (*104*). In hexane extracts these *cis*-peaks generally occur at shorter wavelengths than the 370 mμ peak in the action spectrum (*105*), but this is not necessarily the case in the plant. By analogy with the well-known vertebrate visual system, in which a special "hindered" *cis*-carotenoid derivative is involved, the participation of a *cis*-carotenoid in phototropism is an attractive hypothesis.

TABLE VI

COMPARISON OF ACTION SPECTRUM FOR THE TIP RESPONSE (FIRST POSITIVE CURVATURE) OF *Avena* AND THE ABSORPTION SPECTRUM OF THE HEXANE EXTRACT OF *Avena* TIPS

Peaks of action spectrum (mμ)	Ratio of height to height of principal peak	Peaks of absorption spectrum (mμ)	Ratio of height to height of principal peak
370	0.53	—	0.26
425	0.70	422	0.78
445	1.00	442	1.00
474	0.82	472	0.88

An obvious objection is the absence of a *cis*-peak from the hexane extract. There are several possible explanations for this. In the first place the *cis* isomer might constitute only a very small fraction of the total carotenoid in the extract, so that its *cis*-peak would not be observed, and spectral changes upon I_2-light isomerization would be very minor, as is the case with the present extracts. In the second place, since *cis*-peaks are a consequence of rather special structural configurations, the procedure of extraction might not yield the active *cis* isomer. Support for this argument is given by analogy with the visual system, in which Wald (*106*) has found that, when rhodopsin is split into protein plus retinene, the latter appears mostly in the *trans* form, although it is the "hindered" *cis* form that is combined with the protein. The existence of specific carotenoid-proteins in plants has recently been demonstrated by Nishimura and Takamatsu (*107*). The possibility thus remains open

that the sensitivity peak in the ultraviolet may reflect a particular structural modification of a carotenoid in the photoreceptor complex.

Bünning (*108*) found a close parallel between the distribution of phototropic sensitivity and the distribution of carotenoids in the *Avena* coleoptile, although most of the carotenoid lay in a region just below, rather than at, the extreme tip, whereas Lange's data (*38*) imply that the greatest sensitivity is in the topmost ¼ mm. Because the work depended on histochemical observations, however, we have recently de-

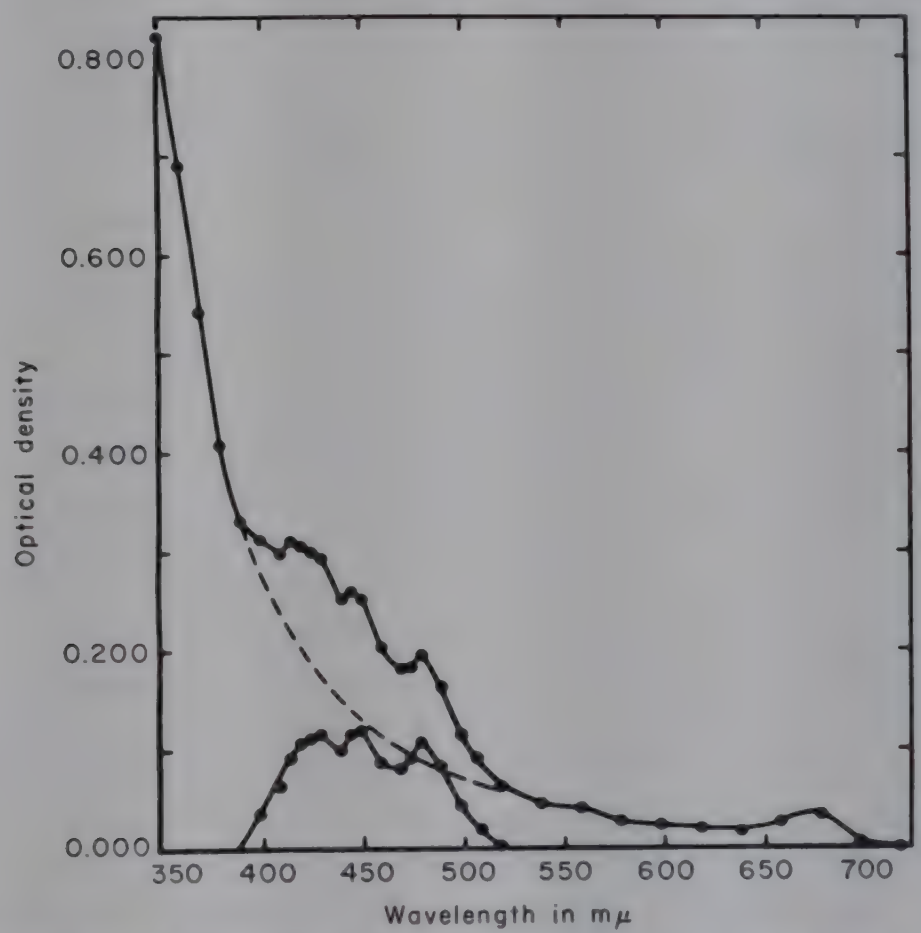


FIG. 10. *In vivo* absorption spectrum of *Avena* coleoptile tips (see text). Upper curve, observed spectrum; lower curve, spectrum corrected for end-absorption (cf. Fig. 8).

termined the *in vivo* absorption spectrum of coleoptile tips using the opalescent plate technique of Shibata *et al.* (*109*). The curve obtained with 32 tips (primary leaves removed) is shown in Fig. 10. Very little chlorophyll is present, judging by the small peak at 675 mμ. The three peaks in the blue region clearly show the presence of carotenoids. The sharp rise below 500 mμ is partly attributable to scattering losses, but in good part to alcohol-soluble "end-absorbing" materials. If a smooth curve is drawn between the 390 and 520 mμ points to approximate this end absorption, subtraction leads to the lower curve in Fig. 10. Unfortunately, the interesting 370 mμ region is completely obscured by the

very steep rise in the curve. These results, however, directly confirm the presence, in the photosensitive part of the coleoptile, of a pigment having light absorption properties close to those indicated by the action spectrum.

A third explanation for the absence of a peak at 360 mμ in the absorption spectrum might be that the curvature at that wavelength is due to fluorescence, that is, the 360 mμ light might elicit the emission of fluorescent light of a wavelength between 425 and 475 mμ. A suggestive example of this is furnished by lumiflavin, a breakdown product of riboflavin, which fluoresces in a wavelength range between 425 and 550 mμ with a maximum at 462 mμ (*110*). As yet there is no direct evidence for or against this possibility, but the large number of fluorescent substances found in plants (e.g., by R. H. Goodwin, *111*) makes it not improbable.

Beside the small discrepancies between absorption and action spectra noted above, a group of experiments involving artificial or natural changes in the carotene content of phototropic organs has led some workers to doubt that a carotenoid can be the photoreceptor. T. W. Goodwin and Lijinsky (*112*) found that *Phycomyces* grown on a medium containing diphenylamine, which reduces the carotenoid level to something like 3% of the normal, retained its phototropic sensitivity. Similarly, Reinert (*113*) found that a strain of *Phycomyces* which only produces carotene when cultured on a medium containing glucose, retained its full phototropic responsiveness when cultured on lactate, in which case it was presumably very low in carotene. In the germinating mycelia of *Puccinia* and *Uromyces*, Gettkandt (*50*) found that the photosensitive hyphal tips contained no visible carotenoid, while the part below the tip, which is not photosensitive, is rich in carotenoids dissolved in oil droplets. Furthermore the correlation between photosensitivity and carotenoid was quite poor; of 18 species studied by Gettkandt and other workers, 7 were photosensitive and contained carotenoid, 5 were insensitive and contained none, but 3 were insensitive although containing carotenoid, and 3 more were fully photosensitive without apparently containing any carotenoid. These last, *Botrytis cinerea*, another *Botrytis* sp., and the uredospore mycelium of *Puccinia glumarum*, deserve further study.

Similar findings have been reported with seedlings. Bandurski *et al.* (*114*) claimed "normal" photosensitivity in albino corn seedlings from which they could extract no measurable amounts of carotene, and Galston and co-workers [cited in (*23*)] have found a similar situation in albino barley coleoptiles. On the other hand, several workers (*22*, *115*) have found that carotenoid is still present in such albino strains, though in small amounts; Virgin's data (*115*) indicate that it is about 5% of the

amount in normal leaves. Turian (*116*) found with a Mycobacterium that diphenylamine never lowers the carotenoid content to less than 5 to 10% of normal. Since quantum yields in a photodynamic system are virtually independent of the concentration of the photodynamic agent (*117*), these arguments cannot be considered final.

Evidence in the opposite sense is furnished by unpublished experiments in our own laboratory with a *Helianthus* mutant which is not only pure white to the eye but which loses its chlorophyll by bleaching on exposure to weak continuous light (thus probably containing excessively low levels of carotene (cf. reference *118*). These plants showed abnormally low phototropic sensitivity. Thus it is difficult to assess the negative claims above, unless one determines: (a) the quantity of carotenoid necessary for light reception and (b) the lower limit of sensitivity of the analytical methods used.

Another argument, of a negative type, against the carotenoid hypothesis is that light absorption by carotenoids has not been shown to affect the auxin system, or in particular to act on indoleacetic acid (IAA). Schuringa (*119*) and Kögl and Schuringa (*120*) reported that carotenoids, either in alcoholic solutions or in aqueous suspensions, could sensitize the photoinactivation of auxin-a-lactone, but these experiments have not been confirmed and the role of auxin a and its lactone as plant hormones is now in doubt. Attempts to find a carotenoid-sensitized photoinactivation of IAA *in vitro* have failed (*121, 122*). In view of the argument above, however, that it is the system producing and transporting auxin which is light-sensitive (rather than the auxin itself), this failure need not surprise us.

In 1949, Galston and Baker suggested that riboflavin, rather than a carotenoid, might be the light receptor in phototropism, and in recent years many investigators have come to believe this. This view developed from Galston's (*123*) discovery that IAA is rapidly inactivated *in vitro* by light when in the presence of riboflavin. The diffusible auxin of *Avena* coleoptiles is similarly inactivated (*99*). The yellow flavins absorb in the same part of the blue region as do the carotenoids (Fig. 9, A), they are present in the phototropically sensitive tissues—in fact universally—and they are known to act photocatalytically in certain systems. Galston and Baker (*124*) found that riboflavin could sensitize the photoinactivation of various enzymes, possibly accounting for the required interference in the auxin production-distribution system during phototropism. *Avena* coleoptiles contain 28–33 μg. riboflavin per gram dry weight,* the concentration being about the same in the tip as in the base (*98*).

* Our own data give 22 μg., which is comparable though probably significantly less.

In the action spectrum the peak in the near ultraviolet suggests participation by riboflavin. Quantitatively, however, it is much smaller than one would expect if riboflavin alone were the photoreceptor, because the absorption peak in the near ultraviolet of riboflavin, and of other common flavin derivatives, is little, if at all, lower than that in the visible. Studies of the spectral sensitivity for the photolysis of IAA in the presence of riboflavin or concentrated *brei* of etiolated pea epicotyls give action spectra closely resembling the absorption spectrum of riboflavin (*98, 121*). The curves rise sharply below 410 mμ to a height at 390 mμ surpassing the 440 mμ maximum in the visible, but this is in direct contrast to the phototropic action spectrum which is almost at a minimum at 390 mμ.

While the action spectrum may thus be considered rather definitely to exclude riboflavin as a photoreceptor, it does not necessarily exclude all flavins. In a paper which appeared long after this review was completed, Harbury *et al.* (*124a*) present absorption spectra for a synthetic isoalloxazine, 3-methyllumiflavin. This compound in certain nonpolar solvents shows 2 peaks and a shoulder in the visible: in CCl_4 the peaks fall at 447 and 474; in benzene, anisole, or mesitylene, at 450 and 476 mμ. The peak in the ultraviolet falls at 333–341 mμ; the ratio of its height to that of the principal peak is about 0.8. Harbury *et al.* consider that the position of the ultraviolet peak depends on hydrogen bonding with the solvent, for in water it moves up to 369, in phenol to 370, and in formamide to 362 mμ. Unfortunately, in such solvents the shift of the ultraviolet peak to longer wavelengths is accompanied by disappearance of the two-peaked structure in the visible, so that it appears not to be possible by any choice of solvent to imitate the action spectrum of phototropism. So far only lumiflavin (6, 7, 9-trimethylisoalloxazine) and its 3-methyl derivative have been reported to show this two-peaked spectrum in the visible, although a few flavoproteins appear to have a shoulder on the long wavelength side of the main peak, at around 480 mμ [see especially Theorell and Åkeson's curve for crystalline "old yellow enzyme," (*124b*)]. The very carefully purified and crystallized glycolic acid oxidase (*124c*) shows no such shoulder. However, it is always possible that further study of the flavins and their derivatives, or of the effects of solvent and of additional solutes on the absorption spectra, might bring to light a combination of substance and conditions giving an absorption spectrum similar to the phototropic action spectrum. Even so, it would still remain to be shown that the substance in question does in fact occur naturally in the plant.

The proposed role of flavins in phototropism is open to question on other grounds. In the first place, many other substances, e.g., eosin,

thionine, quinine sulfate, methylene blue, and even chlorophyll (in fact, almost any fluorescent substance), can act as sensitizers for IAA photooxidation (*78, 121, 122, 125, 126*). Secondly, there is no evidence that riboflavin acts *in vivo*. Thus if the action spectrum for IAA destruction by the *brei* is held to demonstrate photoreception by riboflavin, then the action spectrum for phototropism shows no such participation. In a recent re-examination of these effects Mer (*127*) has found that when the riboflavin content of coleoptiles is increased by providing an external supply, illumination still does not cause any greater decrease in growth than in controls. He points out that "in this experiment riboflavin was known to be present in the coleoptile and the absence of effect must have been due either to a failure of riboflavin to enter the cells or to the absence of a reaction with the naturally occurring auxin." He concludes that while auxin and riboflavin might interact in light *in vivo*, no experimental evidence supports it. Thirdly, the quantum yields and auxin studies discussed earlier indicate that auxin is redistributed, rather than destroyed, in the tip response.

In sum, there are three main criteria for a decision about the probable photoreceptor; the action spectrum, occurrence and distribution in the plant, and possible modes of action. For the tip response in *Avena*, a carotenoid best fits the first two criteria, but there is some *in vitro* evidence to connect a flavin with the last; a final decision between one or the other, or in favor of some quite different, as yet unsuspected, pigment, will only be possible when all three criteria are satisfied. All in all, the balance of evidence is still in favor of the conclusion reached fifteen years ago (*128*): "it may be concluded *tentatively* that the primary process of phototropism is mediated through carotenoid pigments."

There is, perhaps, one possible objection to attaching decisive importance to the action spectrum, namely that the relative effectiveness of different wavelengths might be determined by the light absorption by pigments other than the effective one. This idea, that the action spectrum is a function of "masking pigments" rather than of the active photoreceptor itself, will now be discussed and its possibilities and limitations presented.

C. Interactions between "Active" and "Inactive" Pigments in the Photoreception Process

1. *In Phototropism*

It has been argued recently (*21, 86, 121, 129–131*) that a carotenoid-like action spectrum may be a consequence of a "masking" or "internal

screening" effect of large concentrations of carotenoid pigments in the presence of relatively low amounts of the actual photosensitizing pigment (assumed to be a flavin). Brauner (*21*) has aptly summarized Reinert's main contention as follows: "the high phototropic sensitivity of the tip is explained by the optical filter action of the carotene present, which secures the necessary light absorption across the organ, thus protecting the auxin on the posterior side from destruction. Since the difference in brightness between both flanks is mainly controlled by the internal carotene screen, then the phototropic action spectrum must be determined by the double-peaked absorption curve of the carotene."

Reinert's experiments (*121*), which were largely responsible for this theory, showed that when IAA is inactivated by visible light in the presence of riboflavin and β-carotene, the carotenoid masking introduces two peaks into the action spectrum, not by addition of the carotenoid peaks to that of riboflavin, but by depressing part of the riboflavin curve. He worked with a colloidal suspension of β-carotene in water. This had no action alone as a sensitizer for IAA photolysis. Its absorption spectrum possessed a *single*, broad peak near 420 mμ. The riboflavin, which does sensitize IAA photolysis, had its usual broad peak around 450 mμ, and the action spectrum for riboflavin plus IAA alone gave a sharp peak at 430 mμ [cf. Galston and Baker (*99*), who found a broad peak at 440 mμ for a similar system]. The riboflavin-β-carotene-IAA system gave rise to a two peaked action spectrum for IAA photolysis, one peak being at 430 mμ and the other at 450 mμ. The curve rose sharply below 410 mμ. (This rise was also noted by Galston and Baker in their concentrated *brei* system, but they found no interference, apparently, by carotenoids or other pigments in the system.)

In Reinert's words: "Das Maximum der Riboflavinwirksamkeit bei 430 mμ bleibt auch in Reaktionsgemischen mit Carotin erhalten, weil die benachbarten Messpunkte noch im Bereich des breiten Gipfels der Carotinabsorption liegen. Der zweite Gipfel bei 450 mμ ergibt sich aus der stark ansteigenden Carotinabsorption ab 455 mμ. Der Tiefpunkt bei 410 mμ ist eine Folge der sich verringernden Abschirmung des langwelligen UV-Lichtes durch das Carotin."

Thus the carotenoid absorption depresses the action of riboflavin, presumably by competing for the light. Yet, when the theory is extended to phototropism, the reverse is presumed to occur ("*high* phototropic sensitivity of the tip is explained by the optical filter action of the carotene"). This reversal is apparently connected with the fact that the phototropic effect depends on the *gradient* of light action across the coleoptile.

Let us now consider a simple system equivalent to Reinert's (*34*).

Let a transparent container, one unit thick, contain two pigments, A (active) and M (masking), homogeneously distributed. For monochromatic light incident on this container A and M will have absorption constants a and m (equivalent to optical densities) whose values depend on the concentrations of A and M and on the wavelength of the light (as functions of the latter, the values for a and m will describe the absorption spectra of A and M). If A were present alone the values of a would be specified by the Bouguer-Lambert law (monochromatic light):

$$I = I_0 e^{-a}$$

similarly for m

$$I = I_0 e^{-m}$$

(Since we are assuming unit path length, l does not appear in these expressions.) Since both A and M are present, the intensity at the back of the container will be:

$$I = I_0 e^{-(a+m)}$$

This means that the amount of the incident intensity, I_0, absorbed in the container is:

$$I_0 - I = I_0[1 - e^{-(a+m)}]$$

It can readily be shown [see French and Young (*132*) for a related argument] that A absorbs a fraction $a/(a + m)$ of this amount. Therefore, the amount of light absorbed by the "active" pigment, A (and, proportionately, the amount of photochemical action), is given by:

$$Q_A = I_0 \frac{a}{a + m}[1 - e^{-(a+m)}]$$

Note that the fraction

$$\frac{1 - e^{-(a+m)}}{a + m}$$

is always less than or equal to 1. Therefore, as $a + m$ increases, Q_A always decreases, meaning that M masks A in a negative sense. For very small a and m the term in brackets approaches $a + m$, in which case the action spectrum is independent of M, since Q_A approaches $I_0 a$. For very large m and small a, Q_A approaches $I_0 a/m$, and the action spectrum depends *directly* on the absorption spectrum of A, *inversely* on the absorption spectrum of M; (i.e., negative masking as in Reinert's experiment). It can readily be seen that this is the limiting case for maximum effect on Q_A due to change (with wavelength) in m.

Two simple cases will serve to illustrate these relationships. First, suppose that a is 0.1 (approx. 10% absorption) while m is 0.5 (approx. 40% absorption). Direct substitution in the equation for Q_A will show that A then absorbs 7.5% of the incident light when both materials are present. If, now, m increases by 20% (e.g., when the wavelength is changed), Q_A falls to 7.2% of I_0. Hence, for a and m in this range a 20% rise in M's absorption spectrum will cause only a 4% fall in the action spectrum (assuming A's absorption spectrum is flat in this region). Second, assume that a is 0.0001 and m 5.0 (approx. 99% absorption by m). In this case a 20% rise in m will cause a 20% decrease in Q_A. At such concentrations the basic absorption laws may not be strictly applicable, but the effect of M on A cannot be greater than this.

These relationships give some basis for Reinert's results. However, since he used concentrations of riboflavin comparable with those of carotene, the masking effect to be expected would be more like the former example above, i.e., large changes in masking cause only small changes in action spectrum. This being the case, it is difficult to see from a quantitative point of view exactly how his final curve resulted, although it is not qualitatively unreasonable.

Similar "negative" masking would occur in a nonhomogeneous system where the masking pigment overlies the "active" absorber. Suppose we have two units, the first containing M and the second containing A. In the first unit the intensity, I_0, is attenuated to I_0e^{-m} and in the second unit the fraction $(1 - e^{-a})$ of this is absorbed, so that $Q_A = I_0e^{-m}(1 - e^{-a})$. As m increases Q_A decreases, which is "negative" masking again.

An example of this type is the erythemal spectrum (action spectrum for sunburn), which has a sharp minimum near 280 mμ. To quote Blum (*133*), "The strong absorption by the corneum in the region of 0.28 μ probably accounts to a considerable extent for the minimum of effectiveness of these wavelengths in producing erythema. . . . With a spectrally selective absorbing layer (the corneum) superficial to that in which the photochemical reaction occurs (the Malpighian layer) the measured erythemal spectrum does not reflect directly the nature of the absorbing substance which is concerned in that reaction."

As contrast to the above, let us consider a case where a gradient of light action is involved. Suppose we have a container three units thick, the first unit containing A, the second (center unit) M, and the third (back side) A. In unit 1:

$$Q_{A,1} = I_0(1 - e^{-a})$$

In unit 2:

$$Q_{A,2} = 0$$

but the intensity incident on unit 3 becomes $I_0e^{-(a+m)}$. Therefore in unit 3:

$$Q_{A,3} = I_0e^{-(a+m)}(1 - e^{-a})$$

The *gradient* of photochemical action in this system will simply be the difference in the amounts of light absorption by A in units 1 and 3 (front and back). Thus:

$$\Delta Q_A = Q_{A,1} - Q_{A,3} = I_0(1 - e^{-a})[1 - e^{-(a+m)}]$$

Since for maximum masking a must be small in comparison to m, the expression simplifies to:

$$\Delta Q_A = I_0a(1 - e^{-m}).$$

In this case increases in m evidently increase the gradient of photochemical action. In this sense the idea that the masking pigment "secures the necessary light absorption across the organ" is valid, and any masking in such a system will always be in a positive sense. However, inspection will show that when m is large, M will have relatively little effect on the shape of the action spectrum. Thus, if m changes from 2 to 2.2 (10% increase), the fraction $(1 - e^{-m})$ changes from 0.865 to 0.889, which is less than a 3% increase. Therefore, the supposition that "the phototropic action spectrum must be determined by the double-peaked absorption curve of the carotene" requires careful quantitative study.

Differentiation of ΔQ_A with respect to m (a constant), shows that changes in m have a maximum effect on ΔQ_A when m is small, i.e., when the masking pigment is at a low absolute concentration (although still present in much greater amount than A). The expression for the gradient of light absorption by A then reduces to: $\Delta Q_A = I_0am$ and the action spectrum simply becomes the product of the two absorption spectra. This represents the maximum possible masking effect of M in this system. A 10% increase in m will now cause a 10% rise in the action spectrum, providing a does not change in the same wavelength interval. If the absorption spectrum of A were perfectly flat throughout the range under consideration, the action spectrum would be completely determined by the absorption spectrum of M.

In the phototropic system, however, the absorption of both a and m changes with wavelength, and the gradient is therefore the product of the absorption spectra. In Fig. 11 the product function $(r \times c)$ of a riboflavin absorption spectrum and the absorption spectrum of a typical carotenoid extract is plotted. The extract curve was used in this figure because it gives a better estimate of the postulated masking pigments in the plant than does the absorption spectrum of one particular carotenoid. It is evident that the product curve bears little resemblance to the observed action spectrum. Even the selection of a pure carotenoid

having three sharp peaks in the 400 to 500 mμ range does not lead to an action spectrum that looks anything like the carotenoid, given the above masking arrangement. As stated, this represents the limiting case. It can be shown that the participation of pigments (or scattering processes) other than the carotenoid (and this would undoubtedly not be the only masking substance in the plant) simply reduces the specific effect of the carotenoid on the absorption spectrum of the "active" riboflavin.

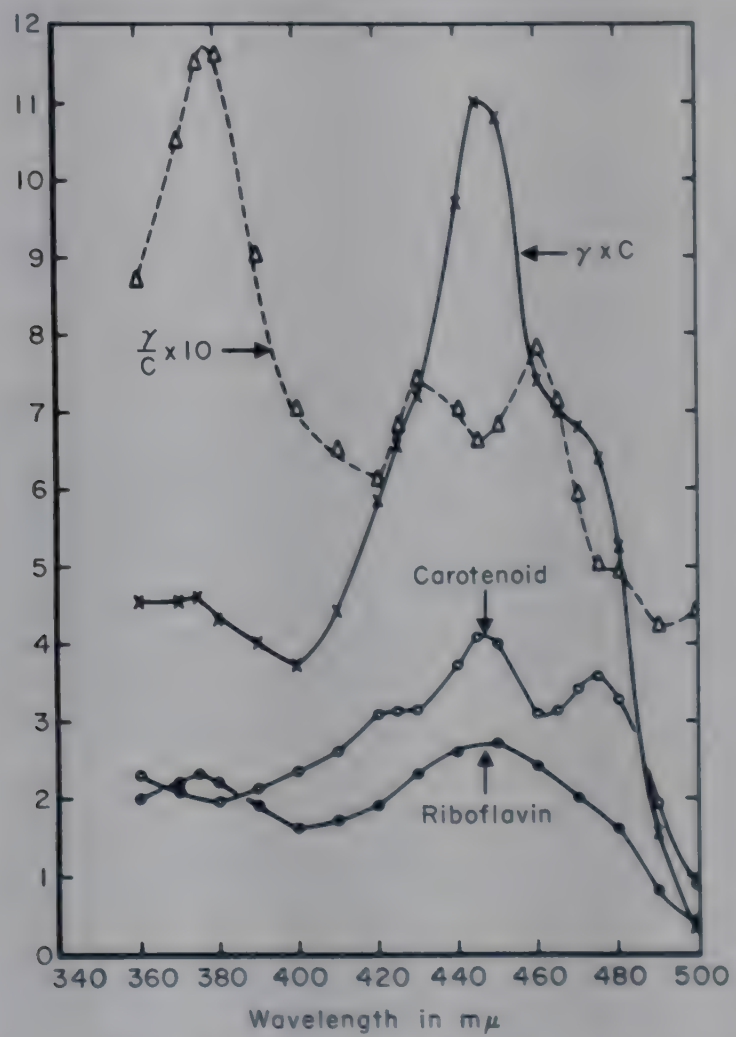


FIG. 11. Action spectra to be expected in limiting cases of masking of "active" riboflavin by "inactive" carotenoid. The absorption spectra of the two components are shown in the lower part of the figure. Ordinate is arbitrary. (From Curry, 34.)

It may be objected that the arrangement examined above is not the best for the demonstration of the masking effect. It can be shown quite directly that in the case of a homogeneous distribution of riboflavin and carotenoid in "front" and "back" halves of the system, the same limits for maximum masking apply. With both pigments in low concentration the limit is the product function $r \times c$; if the carotenoid is in very high concentration the limit is the reciprocal function r/c (i.e., negative

masking). This function has also been plotted in Fig. 11 to show that it, too, cannot account for the observed action spectrum.

The absolute concentrations of riboflavin and carotene in *Avena* are known; the riboflavin content is 20–30 μg. per gm. dry weight (cf. Section VI, B), and Bünning (*131*) estimated that the apical mm. contains 100 μg. lutein per gm. dry weight, enough for about 10% absorption of I_0. It is important to note that over a wide range of intermediate concentrations of riboflavin and carotene like these, the postulated masking of riboflavin by carotene will be very small, because increases in c, though increasing the gradient, at the same time decrease the fraction of I_0 absorbed by riboflavin in *both* sides of the organ.*

In many respects the *A-M-A* arrangement discussed above is just the situation examined in certain of Brauner's (*71, 129*) experiments. He found that the presence of an "inactive" light absorber between the "actively" absorbing regions could augment the phototropic response; e.g., introduction of (100% absorbing) india ink into the hollow of the coleoptile approximately doubled the curvature obtained on four hours' illumination of the base. For processes involving the subapical regions, this effect seems entirely reasonable according to the above interpretation. The gradient of light action is increased, but nothing further is implied about (nor is there any direct evidence for) specific changes in the action spectrum due to the introduction of the dye. Similarly, in the near-ultraviolet, the scattering and absorption losses across the tip will increase with decreasing wavelength and thus increase the gradient; this will distort the observed action spectrum in this region. As the wavelength becomes shorter, the effect of the ultraviolet will be more and more exerted on the front side (cf., Carlile, *134*).

It must be concluded that while the presence of an inactive pigment will increase the gradient it will not "determine" the action spectrum. It can only modify it to a limited extent, even in the extreme case, and will not transform it in the manner proposed by Reinert.

2. *In Phototaxis*

A particular case of a masking or screening action has long been discussed as a basis for phototactic movement. The stigma or other screening material shades the photoreceptor (which is considered to lie at the base of the flagellum) as in the case of *M* overlying *A* above. This might be expected to lead to "negative masking," but in this situa-

* Note that the high phototropic sensitivity of the carotenoid-low mutants described in Section VI, B is impossible to reconcile with the masking theory, because on this theory the sensitivity ought to depend on the gradient imposed by the masking pigment.

tion it is supposed that the organism moves so as to keep the photoreceptor fully illuminated. Hence a change in motion is only possible when light falling on the photoreceptor becomes intercepted by the screening substance. In this case, then, the screening substance acts in a "positive" rather than in a "negative" sense. Change in motion can only occur in regions of the spectrum where *both* the photoreceptor *and* the screening pigment absorb. If, for instance, the photoreceptor were to absorb uniformly over a given spectral region, then the sensitivity would be a *direct* function of the absorption by the masking pigment (and vice versa). In general the sensitivity will thus be a *product* of the absorption of the two pigments. As discussed in Section VI, A, this fact provides one possible basis for the lack of correspondence between the action spectrum and the absorption of any of the pigments known to be present in the cell.

D. Photoreceptor for the "Base Response" of *Avena*

The spectral sensitivity of the "base response" appears to differ from that of the "tip response," and the specific nature of its photoreceptor for visible light remains obscure. This is partly due to the fact that the base response is quite insensitive and difficult to isolate in *Avena*. Haig (33), studying base response as a function of wavelength and using reaction time as a criterion, found an action spectrum rising steadily toward the violet, the highest point occurring near 410 mμ. Haig's curve does not suggest any specific pigment, although both Galston and Baker (99) and Went (22) say that it indicates riboflavin. If it has any recognizable structure it is more like that of a carotenoid, for it has three slight peaks in the blue. Thorning (135), studying the light-growth reactions of decapitated *Avena* coleoptiles, found that 447 mμ light was more effective than 460 mμ, and 460 mμ more effective than 477 mμ, suggesting that the photoreceptor does not have two absorption peaks in this region.

In regard to the base response in the ultraviolet, however, the data are clearer. The action spectrum for ultraviolet-induced base responses shows two peaks at 280 and 297 mμ (see Fig. 12). It is notable that the wavelengths absorbed by riboflavin are relatively ineffective; indeed the 370 and 265 mμ peaks of riboflavin coincide with low points in this action spectrum.

The shape of the action spectrum as a whole is suggestive of that of IAA itself, though slightly modified around the peak and shifted about 12 mμ towards the red. Since also application of IAA to decapitated plants reinstates their ability to curve towards ultraviolet it was suggested (26) that the photoreceptor may be a biologically active complex of IAA. However, other possibilities no doubt exist.

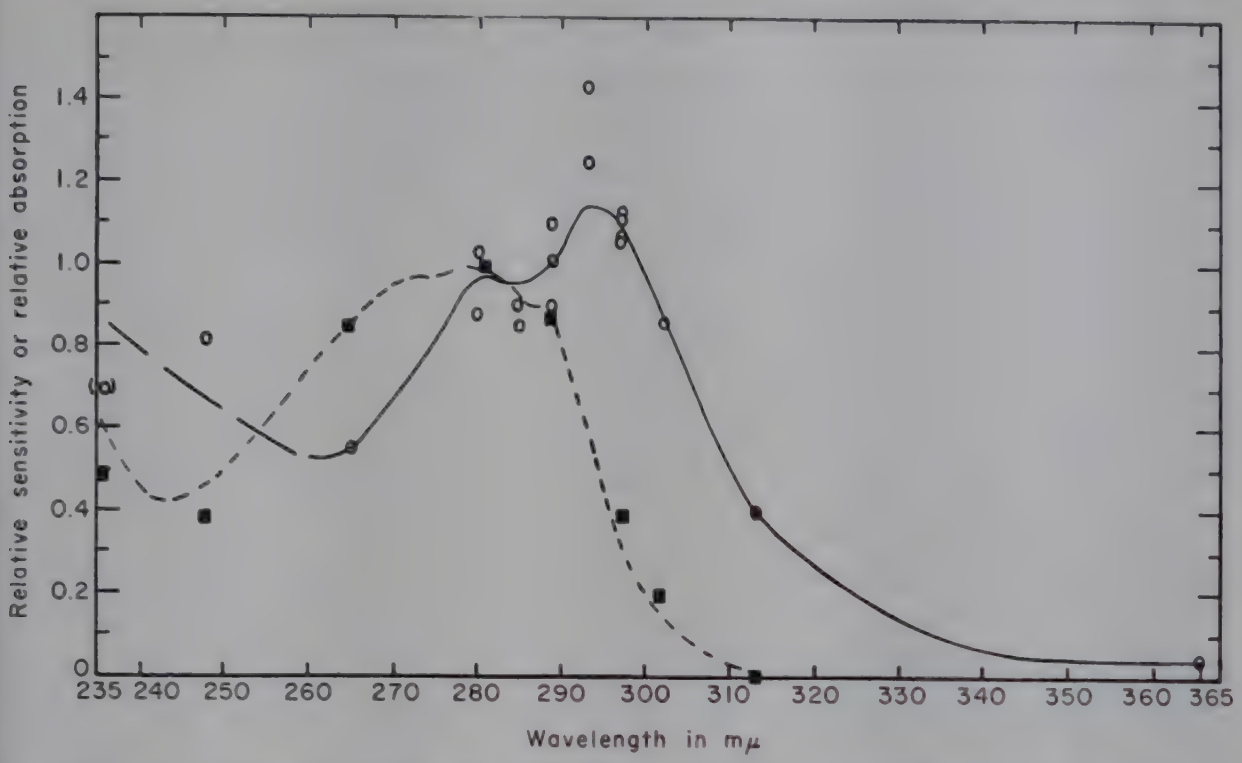


FIG. 12. Action spectrum for the base curvature of *Avena* coleoptiles (circles and solid line). The ordinates give the reciprocal of the number of quanta needed for 8.6° curvature, relative to that at 289 mμ. The dotted curve is the absorption spectrum of IAA, the optical density at 280 mμ having been taken as 1.0. The black squares show the action spectrum for photooxidation of IAA solutions *in vitro*, the value at 280 mμ being taken as 1.0. (From Curry *et al.*, *26*.)

E. PHOTORECEPTORS FOR PHOTOTROPISM IN LOWER PLANTS

There is only limited evidence as to the specific photoreceptors in the phototropism of the fungi. With *Pilobolus*, Bünning (*108*) found two sharply defined peaks at 445 and 485 mμ, while 360 mμ light was only ⅕ as effective as 445 mμ. This spectrum is extremely suggestive of that for *Avena*, and furthermore the peaks agreed precisely with the absorption peaks of the carotenoids which Bünning found to be concentrated in the light-sensitive zone of the *Pilobolus* sporangiophore. Thus in *Pilobolus* also the photoreceptor may well be a carotenoid. It is true that Gettkandt's observations on germ tubes of a number of fungi (*50*) do not particularly support the view that a carotenoid is the photoreceptor, unless, indeed, the functional carotenoid is present in amounts too small to be microscopically visible. However, she did observe that the long wavelength limit in two *Puccinia* species lay between 450 and 480 mμ. Similarly, "colorless" rhizoids of the liverworts *Marchantia* and *Lunularia*, which are negatively phototropic, respond only to blue light, and the wavelength limit lies between 470 and 497 mμ (*49*). Both sets of data indicate a yellow pigment with a sharp cutoff at the long wavelength end.

For *Phycomyces,* Blaauw (*32*) found a single peak near 495 mμ, but his filter combinations only permitted examination of a very few limited spectral regions. Castle (*52*), also using only a few spectral regions, found high sensitivity around 425 mμ and almost none at 525 mμ. A carotenoid is present in the sporangiophores (Castle, 52, and subsequent workers) and indeed is best detected in the growing zone a few mm. below the sporangium (*135a*). A detailed action spectrum for the positive phototropic curvature in visible light, and a less detailed one for the negative curvature in the ultraviolet have just been determined in the authors' laboratory and are shown in Fig. 13 (*135b*). Since the relation between curvature and light dosage in *Phycomyces* is not a simple one (cf. Sections IV, B and IV, C) the action spectra were determined with continuous light, using a balance method against a standard blue light of low intensity. The correspondence between the spectrum in the visible and that for the tip reaction of *Avena* coleoptiles is striking: the peaks at 445, 470, and 370 mμ compare very closely with 445, 474, and 370 listed in Table VI, and the small shoulder around 430 is probably present too. It may be tentatively concluded not only that the photoreceptor in *Phycomyces* is a carotenoid but that it is probably the *same* carotenoid as in *Avena.* Since the two plants are so widely separated systematically, this hints at a limitation in the number of effective photoreceptors in plants.

In the blue-green alga *Tolypothrix,* the erect aerial trichomes curve towards the light, though insensitive to gravity (*74*). The curvature is very slow, 72 hours being required for an experiment. However, by using continuous illumination at relatively low intensities with the active light, and simultaneous illumination from above with phototropically inactive orange light, the culture was kept healthy and an action spectrum could be determined (*74*). It has a peak at about 450 mμ and a shoulder at 470–480 mμ. At 500 mμ and above there is only very slight response. The organism contained (besides chlorophyll a and phycocyanin) a carotenoid believed to be myxoxanthin, with a single broad band at 470 mμ, and β-carotene, which was purified. The peaks of the latter at 450 and about 480 mμ, with a faint shoulder at 420 mμ, were considered (*74*) to identify it as the photoreceptor for this alga.

VII. Mechanisms

A. Variation of Curvature with Dose

We have seen that analysis of curvature and light-growth reactions with respect to the kind of response, time course, sensitivity distribution

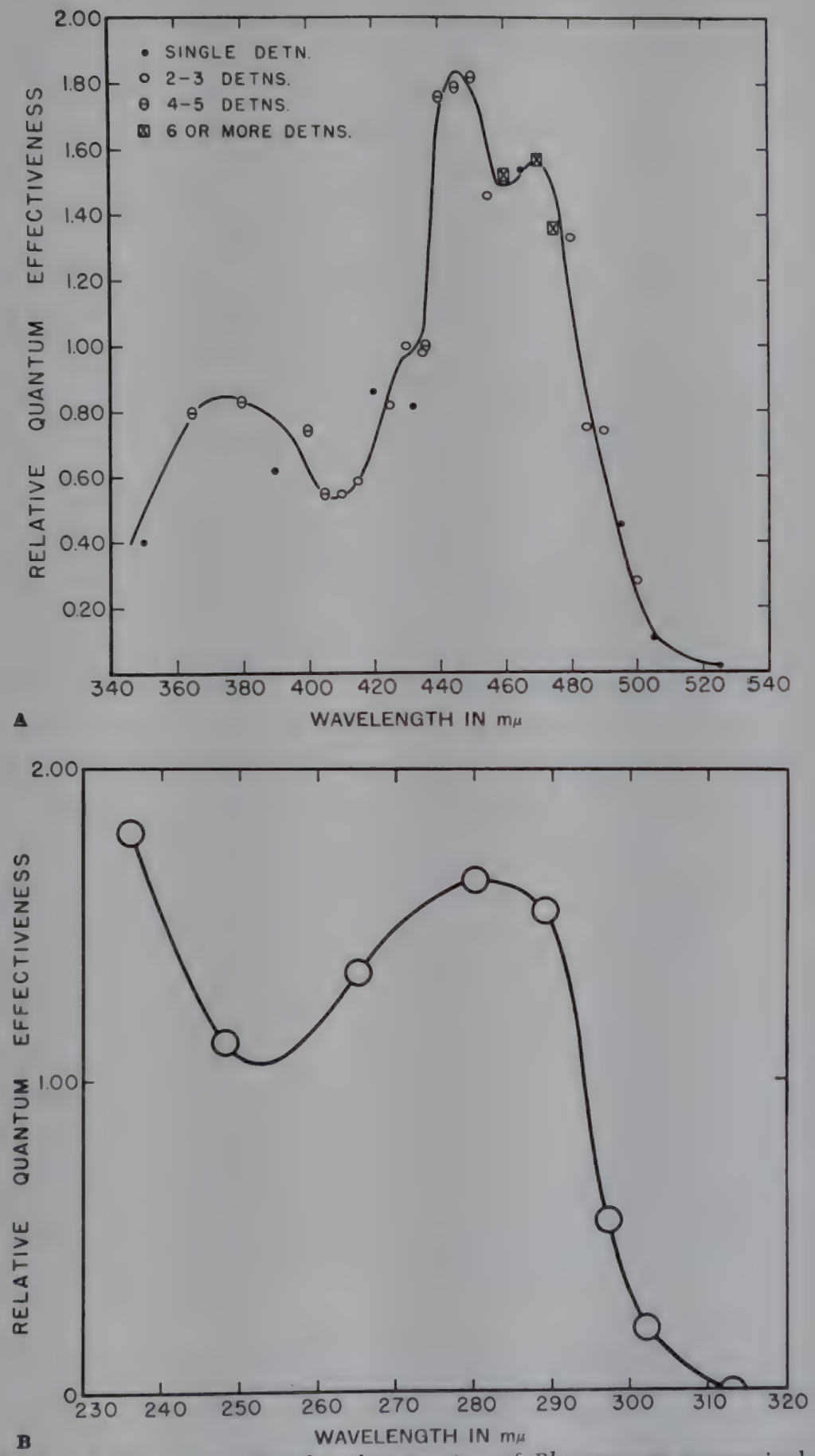


FIG. 13. Action spectra for the phototropism of *Phycomyces* sporangiophores. A, Positive curvature in visible light. B, Negative curvature in ultraviolet. From Curry and Gruen (*135b*).

along the plant, and wavelength sensitivity indicate two phototropic mechanisms in *Avena*. With short exposures the reactions depend, over a range of several log units, on the amount of energy applied ($I \times t$), suggesting that the response is initiated by a simple photochemical change in some light-absorbing substance in the plant. With supra-optimal light doses the response decreases again (Fig. 2), which may be due to a light-saturation phenomenon; i.e., if the photochemical action on the exposed side of the plant is maximal at a certain dose, then increased amounts of light will only serve to push the reaction to completion on the shaded side, thus decreasing the *gradient* of photochemical action and its resultant growth rate redistribution. This can explain the falling off of the response curve, but not the production of negative or second positive curvatures.

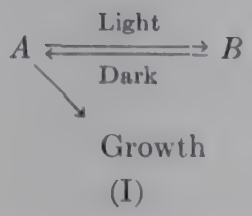
In *Phycomyces* there is a very similar light-saturation effect, as described by Castle (*41*), the result of which is that virtually no curvature takes place at intensities above 1800 MC, if the exposures are longer than 0.6 second. However, this saturation does not account for the large positive curvatures which can take place in continuous light. The discrepancy between these and the light-growth reaction was pointed out on pp. 259–261. The phenomena certainly suggest that there may be a second mechanism accounting for phototropism at high light dosages and long exposures in *Phycomyces*. It was pointed out earlier, however (see Section IV, B), that in the light-growth reactions of *Phycomyces*, Delbrück and Reichart (*45*) have shown the existence of a rapid adaptation phenomenon which, if it occurs in phototropism also, may complicate the interpretation considerably, particularly in regard to curvatures in continuous light (*32*, *136*).

Blaauw (*32*) believed that two opposing light reactions operate in the phototropic system, both in *Avena* and in *Phycomyces*. The first would begin to operate at low doses, the counteraction at high doses. The failure of the reciprocity law at high doses could be attributed to interactions between the two. This idea can account for negative curvatures, but a third mechanism must then be invoked to explain the second positive curvatures. This concept is of historical interest as one of the first interpretations of phototropism invoking a dual mechanism.

B. Base Response

The important features of the base response are: (a) the growing regions are the most sensitive, (b) the reaction appears near the site of light reception, (c) the reaction is relatively quick, and (d) a recovery process occurs, i.e., the curvatures (and the light-growth reactions) are transient. The response to short wavelength ultraviolet pre-

sents the most clear-cut case. As a model we might suppose the system shown (Scheme I).



(I)

where A is a factor (auxin complex?) immediately controlling growth, and B is an inactive form of this.

There are three main reasons for thinking that A may be related to indoleacetic acid. In the first place, as mentioned in Section VI, D, the ultraviolet action spectrum, though slightly modified and shifted about 12 mμ towards longer wavelengths, is similar to the absorption spectrum of IAA (Fig. 12). Secondly, IAA is known to be photoinactivated by ultraviolet in this part of the spectrum, and with quantum yields approaching unity (cf. pp. 267–8). Tip diffusates are similarly inactivated (see Section V). However, the amount of base curvature resulting from a given dosage of ultraviolet is clearly greater than would be expected if the ultraviolet were inactivating IAA in simple solution. It has been proposed (*26*) that the IAA may be in the form of an ultraviolet-sensitive complex, but more probably the light action is not exerted directly on the auxin but on some mechanism in the cell which transports, or reacts with, the auxin. Lastly, plants which have been repeatedly decapitated become phototropically insensitive to ultraviolet, but the sensitivity is largely restored by applying IAA in agar blocks before exposure to ultraviolet (or to very large amounts of blue light).

The idea of a back reaction from B to A arises from the transient nature of the base response; i.e., growth changes produced by light are compensated by a subsequent dark reaction (cf. the "anti-reaction" of Burckhardt *137*). This might account for failure of the $I \times t$ relationship with long exposures. Curvature would depend not only on how much A is in the inactive B form, but also on the time it remains in that form.

It was assumed above that B is inactive. However, the experiments of Meyer discussed below show that the photolysis products of IAA (albeit produced with excessively high light doses) actually inhibit growth. If this mechanism participates in normal phototropism the compensating anti-reaction might be due only to the removal of B.

The base response to visible light requires much more energy than similar responses to ultraviolet. Possibly this is because A absorbs very little visible light, requiring a sensitizing pigment. The fact that exposures at least 4 minutes long are required in order to observe a base response (curvature) to visible light 90 minutes later may simply be

due to complete compensation of the small light-growth reactions during the 90 minute development period. The light-growth reactions observed on symmetrical illumination by Went (*53*) and van Dillewijn (*39*) substantiate this view; these workers observed definite light-growth reactions when the total energies and exposure times were much less than those required to obtain base curvatures after 90 minutes. These light-growth reactions consisted of a growth rate deceleration followed by an acceleration, the cycle being completed in 90 minutes. Similarly, Haig (*33*) observed threshold base (curvature) responses at much lower doses and after short exposures. Significantly, however, what he observed was the first perceptible tilting of the coleoptile after the stimulus; this occurs in less than 15 minutes, just as the decelerating light-growth reaction begins.

The above facts are consistent with the suggested mechanism. With continuous unilateral exposures some *A* would always be in form *B*, and, if there is a gradient of intensity across the organ, the level of *A* on the exposed side will constantly be less than on the shaded side, accounting for the large, continuously developing curvatures observed with such exposures.

As far as the biochemistry of the conversion of *A* to *B* is concerned, the only theoretical basis comes from the attempts to implicate riboflavin. Galston *et al.* (*138*) showed that catalase inhibits the destruction of IAA by an oxidase preparation from pea stems, and that blue light partly reverses this inhibition. The enzyme itself shows light activation (*139*) which had been previously ascribed to the light reversal of the effect of a natural inhibitor. They therefore proposed that IAA is destroyed by a "light activable flavoprotein enzyme coupled through H_2O_2 to a peroxidase." However, Kenten (*140*) could find no trace of flavoprotein in the preparation, and showed that the "IAA oxidase" consists of a peroxidase similar to that from horseradish (in agreement with Galston *et al.*) plus a soluble heat-stable factor which is not a flavin and which can be replaced by monophenols, aniline, resorcinol, or maleic hydrazide, most of which are peroxidase substrates. The light activation thus evidently depends on something other than the intervention of a flavin. Nevertheless, Andreae (*141*) found that riboflavin (Rbf) could activate what may be a related system, namely the photo-induced oxidation of Mn^{2+} to Mn^{3+}, which he considered was brought about through the conversion of a monophenol or amine to an activated state:

$$\mathrm{Rbf} + h\nu \rightarrow \mathrm{Rbf}^*$$

$$\mathrm{Rbf}^* + \mathrm{M(red)} \rightarrow \mathrm{RbfH_2} + \mathrm{M(ox)}$$

$$\mathrm{M(ox)} + 2\ \mathrm{Mn^{2+}} + 2\ \mathrm{H^+} \xrightarrow{\text{pyrophosphate}} \mathrm{M(red)} + 2\ \mathrm{Mn^{3+}}\ \text{pyrophosphate}$$

where M is a monophenol in reduced (red) or oxidized (ox) state. Subsequently Waygood *et al.* (*142*) concluded that the phenol-activated peroxidase functions in IAA destruction by converting Mn^{2+} to Mn^{3+} which initiates the oxidation and decarboxylation of IAA, and have proposed this as the mechanism of phototropism (*143*). Their enzyme, obtained from wheat leaves, is apparently a peroxidase, and it oxidizes IAA in presence of Mn^{2+} and a monophenol. This is strongly inhibited by riboflavin (2.1×10^{-4} *M*) in the dark. Blue or white light completely reverses the inhibition. They conclude that riboflavin in the *Avena* coleoptile protects IAA from oxidation by the Mn-phenol-peroxidase system, but that light removes this protection: "the photoreversible retarding effect of riboflavin on the reaction provides an interesting and highly probable explanation for phototropic curvatures."

It is difficult to evaluate all these proposals. The inhibition of IAA oxidation by riboflavin found by Maclachlan and Waygood appears to conflict with Galston and Baker's data. The concentration of riboflavin used (2.10^{-4} *M*) is about 100 times that present in the *Avena* coleoptile. The levels of riboflavin in pea, bean, corn, and lupine seedlings, of around 1 μg. per gm. fresh weight or 2.5×10^{-6} *M*, agree well with the *Avena* figure (*144, 145*) so there is some reason to doubt the extent to which the proposed mechanism might operate in plants as a whole. The *in vivo* significance of the reaction is not quite clear, since the addition of riboflavin does not promote the growth of *Avena* coleoptiles in the dark (*127*). Then, too, the *in vivo* significance of the Mn-phenol-peroxidase system is also far from clear. The powerful and specific IAA-destroying enzyme from the fungus *Omphalia flava* is active after exhaustive dialysis, shows only moderate stimulation by Mn and little or none by monophenols (*146*). Spectrographic analysis reveals little or no Mn present. It appears to be an oxidase-peroxidase like that acting on tryptophan. Admittedly, this enzyme is from lower rather than higher plants, but there is good reason to believe it is effective under physiological conditions (*147*). All in all, the biochemical mechanisms so far proposed for light-growth reactions leave much to be desired. The indoleacetic acid oxidase system has recently been reviewed by Ray (*147a*).

Whatever the mechanism is, it might perhaps provide a basis for phototropic curvatures in stems exposed to continuous daylight and perhaps also for those in roots. Such curvatures are mainly of the localized type and are characterized by a relatively high energy (or continuous exposure) requirement; indeed, their relative "insensitivity" is perhaps the main reason they have been so little studied. Wherever auxins are limiting growth, a mechanism of the kind postulated on p. 295 would yield

a light-growth reaction and phototropic curvature if the light is distributed asymmetrically.

The weakness of all such proposals is that they require the auxin to be present at the same time as the illumination. Many years ago Brauner and others (see reference *19* for review) showed that if unilluminated tips were placed on previously illuminated coleoptile stumps the latter would curve, i.e., the effect of the light persists in the tissue in the subsequent dark period. Recently von Guttenberg (*147b*) has greatly extended this type of experiment; coleoptile stumps were exposed to about 7.10^6 MCS of white light, then again decapitated and placed in the dark and indoleacetic acid 0.5 mg. per liter subsequently applied symmetrically in an agar block. Large curvatures resulted. Even when the blocks were not applied until after 8 hours in darkness, curvatures could still result; after 12 hours the effect of the light had finally disappeared. These experiments further increase the improbability that the action could be a direct photoinactivation; it is difficult to believe that photoproducts capable of reacting with and destroying IAA would persist for hours. The polarity of an auxin-transporting system, however, might well persist in the absence of anything for it to transport.

C. Tip Response

To account for the apparently high quantum yields involved in the tip response in terms of auxin redistribution, we need to postulate some kind of amplifying mechanism. It has recently been shown (*148*, *149*) that the products of IAA destruction by bright light inhibit the growth of *Avena* coleoptile sections. The experiment, in principle, was to apply an agar block containing IAA which had been illuminated with riboflavin, (or pure compounds which have been identified as among the photolysis products), and after two hours to replace it with another block containing IAA. The subsequent growth was less than that of controls which had been given either water or unilluminated IAA during the first two hours. If, however, similar experiments were done with sections floating on solution no such inhibition was observed. It was therefore suggested that the photolytic products act by inhibiting the transport of IAA through the section. Such an inhibition of transport by the breakdown products of IAA might provide the required amplification of the light effect. Unfortunately the light energies required to cause accumulation of an inhibiting level of the photolytic products are several orders of magnitude greater than those causing the tip response. Even with added riboflavin as sensitizer 9.10^6 MCS were used in the experiment cited (*149*). In sections illuminated directly, Meyer points out

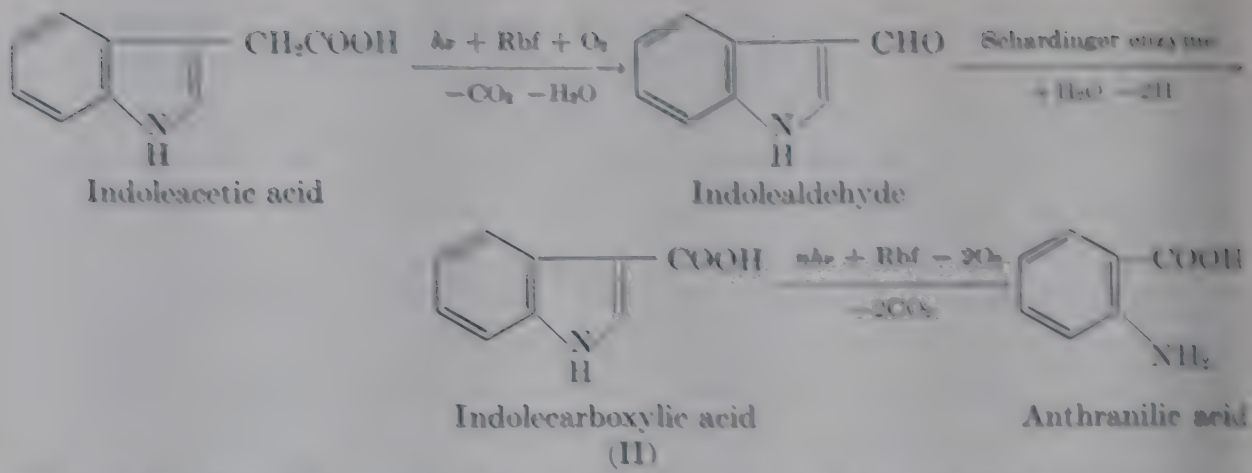
that 100 meter-candles for five hours ($= 1.8 \times 10^6$ MCS) was "not yet effective" in causing growth inhibition. (The tip response is optimal at about 200 MCS; see Fig. 2). Unless it can be shown that auxin transport inhibitors are photolytically formed with much greater efficiency in the tip than elsewhere, we must look for some other and more powerful type of amplification to explain the tip response.*

The simplest expedient is the broad assumption that *the activity of an enzyme in the auxin production-transport system is changed by light.* A possible parallel may be found in the effect of ionizing radiations on an auxin-forming enzyme (*150*). In phototropism the light must be absorbed by a pigment, either intimately associated with the enzyme or capable of direct interaction with it. We have already seen that the decreasing phase of the response curve can be attributed to light-saturation. As in the "base response" mechanism, we must then assume a back reaction, regenerating the pigment (or enzyme), to account for the large tip curvatures which develop under continuous low-intensity exposures.

An explanation for negative curvatures is called for. Like the positive tip curvatures, the ultimate effector of these is an auxin redistribution (cf. Table IV). Therefore the same pigment system is probably involved. The most probable assumption is that the negative curvatures are due to an irreversible bleaching of the photoreceptor pigment at sufficiently high intensities, before reaction with the enzyme. This would simultaneously explain the intensity requirement for negative curvatures and the fact that smaller curvatures are obtained with continuous exposures to such intensities (cf. Table I).

A quite different explanation has been advanced by Meyer and Pohl (*148*) according to which light converts IAA to inhibitory breakdown products which are themselves photosensitive and give rise to compounds which are not inhibitory. Unfortunately one of the best established intermediates (*151*) in the photolytic sequence is indole-3-aldehyde, which is not in the least photosensitive even in presence of riboflavin. However, it is readily converted by Schardinger enzyme, which is known to be present in the plant, to indolecarboxylic acid, which is again photosensitive and breaks down to anthranilic acid and eventually to oxalic and formic acids and glycine (see Scheme II).

* *Note added in proof*: Since the above was written, another example of this (very common) lack of appreciation of the low light dosages effective in phototropism has come to hand in the paper of Fang *et al.* (*149a*) who found a 20% increase in the amount of IAA decomposed in corn shoots after exposure to about 13 million MCS, and ascribe significance to this for the explanation of phototropism.



The data showed that indolealdehyde and indolecarboxylic acid, but not anthranilic acid, inhibited IAA-induced growth. Thus with certain light dosages there would be more inhibitor present on the lighted than on the shaded side, giving positive curvatures, but as the light dosage increases the sequence of reactions would go further and the compounds on the lighted side hence become inactive, so that there would now be an excess of inhibitor on the shaded side. This scheme thus provides an ingenious explanation for negative curvatures.

The enzymatic and photolytic mechanisms suggested are biochemical models, but the observed auxin redistribution might also be accounted for in terms of changes in certain physical properties, such as electrical polarity (*152*) or viscosity (*94*). Interestingly enough, light-induced changes in protoplasmic viscosity and protoplasmic streaming also show change of sign with increasing intensity, and have a wavelength sensitivity similar to that of phototropism (*94, 153*). Furthermore, the rate of streaming is exceedingly sensitive to auxin, being accelerated by low concentrations and decelerated by high (*154, 155*). It is not unreasonable, therefore, to believe that the effect of light on streaming may be exerted through an action on auxin production-transport.

The tip response as seen in *Avena* is probably typical of phototropic responses arising in special auxin production centers. The effect of light on leaf movements is an example. Here, when part of a leaf blade is illuminated and the remainder in shadow, the petiole curves so as to bring more of the blade into the light, i.e., that side of the petiole most nearly in contact with the shaded part of the leaf grows more than the side in contact with the illuminated part. As in phototropism, so also in these "euphotometric" leaf movements (*156*) the effector is a redistribution of auxin between the two sides of the petiole, as shown by Brauner and Vardar (*157*) for *Tropaeolum*, and by Yin (*158*) for *Carica papaya*. The similar reaction in *Coleus* can be imitated by applying auxin asymmetrically to the leaf blade (*159*). According to Yin (*158*) it is the conduction of auxin along the vascular bundles which enables the

asymmetry of auxin production in the blade to be so precisely transferred to the petiole.

Thus, in most plants we may conceive of two phototropic mechanisms, one in which light acts on the source of auxin, and the other in which light acts directly, either on auxin or on some system which combines with auxin, near the point of its utilization in growth. A complete biochemical interpretation of phototropism awaits, on the one hand, a more precise identification of the light-sensitive systems and, on the other hand, further information about the production and transport of auxin and the initial stages of the growth process.

D. The Relation between Light Energy Input and the Response

In the very early work on phototropism the prevailing notion was that the magnitude of the response initiated by light bore little relation to the actual energy input (*Stimmungs-Theorie*). Then both Loeb for animals and Blaauw for plants made it quite clear that, for very small responses, at least, the response is closely dependent on the amount of energy administered (*Reizmengengesetz*). This finding has led some to think that the light energy is itself the source of the energy required for the directed movement. In those phototactic movements, such as that of *Rhodospirillum*, which depend upon chlorophyll and therefore upon photosynthesis, it seems conceivable that the light energy could ultimately be converted to energy of motion. An examination of phototropism and phototaxis in the more typical, sensitive cases, however, indicates that the translational energy must actually derive from the potential energy within the organism; i.e., from the organism's own energy reserves.

In the higher plants we have seen that some form of auxin seems to be implicated in phototropism. Auxins are known to be catalytic in their action; i.e., each hormone molecule controls thousands of growth "events." In the relatively simple base response the action of light on auxin or on an auxin complex may be a stoichiometric photooxidation. Even in this case, however, the resulting redistribution of growth represents a very large amplification of the light energy input, because the small change in the numbers of auxin molecules results in a very large change in the relative volumes of tissue on the two sides of the plant. In the tip response, where light is presumed to act on an enzyme in the auxin production-transport system (Section VII, C), the amplification is even greater because it is two-staged. In the first stage each quantum of light affects the distribution of more than one auxin molecule, and in the second stage each auxin molecule affects more than one growth "event." Thus, an exceedingly small light energy input secures a spatial

redistribution of the processes consuming normal metabolic energy in growth.

In connection with the idea of a light-induced lateral transport of auxin, Went (*160*) calculated that 0.001 erg is required for a lateral transport of auxin in the tip sufficient to give curvature (10^{-13} moles of auxin displaced 1 mm.). He says (*22*) "the minimal amount of light energy required for such a curvature is about 1 erg. This fact makes it possible to view the *induction* of phototropic curvature as a normal physicochemical reaction, for which the energy is supplied by the exciting light" (italics ours). There can be no doubt that light initiates the process by a normal photochemical reaction, but the above considerations indicate that *after the induction* the energy for curvature *development* comes from the normal growth processes.

A simple way of visualizing this induction would be to assume that the absorbed light acts photoelectrically to give a potential across the tip and thus to cause migration of auxin ions. Schrank (*161*) observed transverse electrical polarities of the order of 10 to 20 millivolts induced by 200 MCS unilateral illumination, giving some basis for the photopotential notion. According to Koller (*162*) the photoelectric process is rarely more than 1% efficient, but if we assume for the moment that each quantum absorbed gives one electron, then, since 1 erg of 450 mμ light contains approximately 2.2×10^{11} quanta, 1 erg absorbed would lead to the emission of 2.2×10^{11} electrons. In the most efficient possible process, then, this effect could lead to the displacement of 2.2×10^{11} molecules of auxin. It is interesting that this approaches the required magnitude ($\sim 10^{11}$ molecules; cf. pp. 266–267), but it must be noted that this assumes 100% efficiency, the absorption of 1 erg, rather than the 0.01 ergs actually required, and the participation only of the ions of indoleacetic acid and not of those of any other acid in the cells. Taking these factors into consideration, a more realistic estimate could not exceed the displacement of 10^7 molecules of auxin. In any event both this case and Went's interpretation still invoke the lateral displacement of auxin, whose subsequent action, as we have already noted, is catalytic.

In connection with the photoelectric effect it is interesting to note Schrank's (*163*) finding that direct current of 5 to 20 microamperes applied transversely for 2 minutes to the 5 mm. apical segment of the *Avena* coleoptile induces bending toward the positive pole. The charge transferred here is of the order of 10^{-3} coulombs or (since $e = 1.6 \times 10^{-19}$ coulombs) roughly 6×10^{15} electrons, which is several orders of magnitude greater than the numbers of electrons discussed above.

A different comparison of the energy magnitudes involved may be derived from studies originated by Heyn (*164*) and by Söding (*165*) on

the mechanical properties of bending seedlings. The later data of Tagawa and Bonner (*166*) with the *Avena* coleoptile show that a 220 mg. weight suspended near one end of a 20 mm. section introduces a permanent 22° bend in the section. In the course of the bending the weight moves approximately 1 cm., so that the work done is at least $0.2 \times 1 \times 980 = 200$ ergs. This mechanical bending may seem far removed from phototropic curvatures, but it must be recalled that auxin is thought to act by modifying the mechanical properties of the growing cell wall. Since *at most* only 1 or 2 ergs (approx. 200 erg/cm.^{2} on 1 mm.^{2}) of absorbed light energy are needed to give curvatures of about 22°, it is apparent that the light could in no sense "push" or "pull" the organism nor could it contribute sufficient energy in any photochemical process to accomplish such a deformation.

So far our discussion has been concerned with light-induced *spatial* redistributions of growth. The same conclusion, namely, that the light energy itself does not provide the energy for growth redistribution, may be drawn from an examination of a somewhat different effect, the light-growth reaction. In this case the almost doubled growth rate produced by symmetrical light-stimulation of a *Phycomyces* sporangiophore is compensated for by a subsequent fall below the normal rate (Fig. 5), so that there is essentially a *temporal* redistribution of growth. Hence Delbrück and Reichardt (*45*) conclude that "The stimulus does not produce extra growth, it simply alters the distribution in time of the growth that would have taken place during the same period in the absence of the stimulus. The illumination program controls the rate of utilization of materials available for growth, but does not alter the quantity of this material."

Thus, although none of the evidence is perhaps wholly conclusive, the totality of it points clearly to the use of a small amount of light energy, both in tropism and in taxis, to channel a relatively large amount of metabolic energy into a modification of growth or movement.

E. Summary

If an attempt were to be made to summarize the large amount of work carried out on such a variety of biological material one could only point to the emergence of a very few general principles. These are: (1) the fact that practically all organisms studied are to some extent light-sensitive; (2) the evident occurrence of *more than one* response to light in each of the cases analyzed; (3) the high probability that in virtually all organisms carotenoids participate in at least one of these responses; (4) the almost completely unbridged gap between the reception of light and its first biochemical effect, whether evidenced as change of growth,

of auxin concentration, or of flagellar movement; (5) the strong hint that there is a parallel in the mechanisms of the different responses in taxonomically distant groups (e.g., translocated versus local responses); (6) the principle that the biochemical work done in the response is greater than the light energy absorbed.

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References

1. A. Wetzel, *Z. wiss. Zool.* **143,** 77–125 (1933).
2. H. Laurens, *Bull. Museum Comp. Zool. Harvard Coll.* **53,** 253–302 (1911).
3. P. Halldal, *Physiol. Plantarum* **11,** 118–153 (1958).
4. A. Trembley, "Mémoires pour servir à l'histoire d'un Genre de Polypes d'eau douce." J. and H. Verbeek, Leiden, Holland, 1744.
5. J. Loeb, "Forced Movements, Tropisms and Animal Conduct." Lippincott, Philadelphia, 1918.
6. M. Rose, "La question des tropismes," 469 pp. Presses Universitaires de France, Paris, 1929.
7. W. E. Garrey, *J. Gen. Physiol.* **1,** 101–125 (1918–1919).
8. G. Viaud, *Bull. biol. France et Belg.* **74,** 249–308 (1940).
9. G. P. Adams, *Am. J. Physiol.* **9,** 26–34 (1903).
10. S. O. Mast, *J. Exptl. Zool.* **27,** 367–390 (1919).
10a. P. Halldal, *Nature* **179,** 215 (1957).
11. J. Loeb and H. Wasteneys, *J. Exptl. Zool.* **19,** 23–35; **20,** 217–236; **22,** 187–192 (1915–1917).
12. J. Loeb and J. H. Northrop, *Proc. Natl. Acad. Sci. U. S.* **3,** 539–544 (1917).
13. W. L. Dolley, Jr., *J. Exptl. Zool.* **56,** 185–191 (1930).
14. S. O. Mast, *J. Exptl. Zool.* **38,** 109–205 (1923).
15. D. Kennedy and R. D. Milkman, *Biol. Bull.* **111,** 375–386 (1956).
16. H. G. duBuy and E. L. Nuernbergk, *Ergeb. Biol.* **9,** 358–544 (1932); **10,** 207–322 (1934); **12,** 325–543 (1935).
17. J. van Overbeek, *Rec. trav. botan. néerl.* **30,** 537–619 (1933).
18. H. G. duBuy, *Rec. trav. botan. néerl.* **30,** 798–925 (1933).
19. F. W. Went and K. V. Thimann, "Phytohormones." Macmillan, New York, 1937.
20. W. F. F. Oppenoorth, *Rec. trav. botan. néerl.* **38,** 287–372 (1941).
21. L. Brauner, *Ann. Rev. Plant Physiol.* **5,** 163–182 (1954).
22. F. W. Went, *in* "Radiation Biology" (A. Hollaender, ed.), Vol. 3, pp. 463–478. McGraw-Hill, New York, 1956.

23. A. W. Galston, *in* "Handbuch der Pflanzenphysiologie" (W. Ruhland, ed.), Vol. 17, 492–529. Springer, Berlin, 1959.
24. P. A. deBouter, cited by F. A. F. C. Went, *Koninkl. Ned. Akad. Wetenschap. Proc.* **25,** 475 (1929).
25. D. B. Idle, *J. Exptl. Botany* **7,** 347–361 (1956).
26. G. M. Curry, K. V. Thimann, and P. M. Ray, *Physiol. Plantarum.* **9,** 429–440 (1956).
27. V. L. Steinke, *Planta* **30,** 757–779 (1940).
28. V. I. Palladin, *in* "Plant Physiology" (B. E. Livingstone, ed.), 3rd ed., p. 281. Blakiston, Philadelphia, 1926.
29. C. Darwin, assisted by F. Darwin, "The Power of Movement in Plants." John Murray, London, 1880.
30. F. Czapek, *Jahrb. wiss. Botan.* **32,** 175–308 (1898).
31. N. Ohno, *Jahrb. wiss. Botan.* **45,** 601–643 (1908).
32. A. H. Blaauw, *Rec. trav. botan. néerl.* **5,** 209–372 (1909).
33. C. Haig, *Biol. Bull.* **69,** 305–324 (1935).
34. G. M. Curry, "Studies on the Spectral Sensitivity of Phototropism," pp. 1–152. Ph.D. Thesis, Harvard University, Cambridge, Mass., 1957.
35. O. L. Clark, *Z. Botan.* **5,** 737–770 (1913).
36. W. H. Arisz, *Rec. trav. botan. néerl.* **12,** 44–216 (1915).
37. W. R. Briggs, *Plant. Physiol.* **33,** Suppl. xxii, Proc. Am. Soc. Plant Physiol. (1958); and personal communication.
38. S. Lange, *Jahrb. wiss. Botan.* **67,** 1–51 (1927).
39. C. van Dillewijn, *Rec. trav. botan. néerl.* **24,** 307–581 (1927).
40. E. S. Castle, *Am. J. Botany* **29,** 664–672 (1942).
41. E. S. Castle, *J. Gen. Physiol.* **15,** 487–489 (1932).
42. W. Reichardt and D. Varju, *Z. Physik* **15,** 297–320 (1958).
43. G. M. Curry and H. E. Gruen, *Nature* **179,** 1028–1029 (1957).
44. R. Cohen and M. Delbrück, *J. Cellular Comp. Physiol.* **52,** 361–388 (1958).
45. M. Delbrück and W. Reichardt, *in* "Cellular Mechanisms in Differentiation and Growth," 14th Growth Symposium (D. Rudnick, ed.), pp. 3–44. Princeton University Press, Princeton, N. J., 1956.
45a. E. S. Castle, *J. Gen. Physiol.* **42,** 697–702 (1959).
46. A. H. Blaauw, *Z. Botan.* **6,** 641–703 (1914); **7,** 465–532 (1915).
47. J. Buder, *Ber. deut. botan. Ges.* **36,** 104–105 (1918).
48. E. S. Castle, *J. Gen. Physiol.* **17,** 49–62 (1933).
49. M. Dassek, *Beitr. Biol. Pflanz.* **26,** 125–200 (1939).
50. G. Gettkandt, *Wiss. Z. Martin-Luther-Univ., Halle-Wittenberg* **3,** 691–710 (1954).
51. H. Ziegler, *Planta* **38,** 474–498 (1950).
52. E. S. Castle, *Cold Spring Harbor Symposia Quant. Biol.* **3,** 224–229 (1935).
53. F. W. Went, *Koninkl. Ned. Akad. Wetenschap., Proc.* **29,** 185–192 (1925).
54. N. Cholodny, *Biol. Zentr.* **47,** 604–626 (1927).
55. F. W. Went, *Rec. trav. botan. néerl.* **25,** 1–116 (1928).
56. R. D. Asana, *Ann. Botany (London)* [N.S.] **2,** 955–957 (1938).
57. M. Wilden, *Planta* **30,** 286–289 (1939).
58. W. R. Briggs, R. D. Tocher, and J. F. Wilson, *Science* **126,** 210–212 (1957).
59. P. Boysen-Jensen, *Kgl. Danske Videnskab. Selskab, Biol. Medd.* **13,** 3–31 (1936).
60. P. Boysen-Jensen and N. Nielsen, *Planta* **1,** 321–331 (1925).

61. P. Boysen-Jensen, *Planta* **5,** 464–477 (1928).
62. H. G. duBuy and E. L. Nuernbergk, *Koninkl. Ned. Akad. Wetenschap., Proc.* **33,** Pt. 1, 542–556 (1930).
63. H. Söding, "Die Wuchsstofflehre." Thieme, Stuttgart, 1952.
64. E. Bünning, *Planta* **27,** 583–610 (1937).
65. K. V. Thimann and J. Bonner, *Proc. Natl. Acad. Sci. U. S.* **18,** 692–701 (1932).
66. H. J. Reisener, *Z. Botan.* **46,** 474–505 (1958).
66a. A. W. Galston, *Botan. Rev.* **16,** 361–378 (1950).
67. P. R. Burkholder and E. S. Johnston, *Smithsonian Inst. Publs., Misc. Collections* **95,** 1–14 (1937).
68. C. Brown, *in* "The Physiology of Forest Trees" (K. V. Thimann, ed.), pp. 511–525. Ronald Press, New York, 1958.
69. E. Bünning, H. J. Reisener, F. Weygand, H. Simon, and J. F. Klebe, *Z. Naturforsch.* **11b,** 363–364 (1956).
70. S. A. Gordon and M. Eib, *Plant Physiol.* **31,** Suppl. xiv, Proc. Am. Soc. Plant Physiol. (1956).
71. L. Brauner, *Symposia Soc. Exptl. Biol.* **11,** 86–94 (1957).
72. B. B. Stowe and T. Yamaki, *Ann. Rev. Plant. Physiol.* **8,** 181–216 (1957).
73. C. B. van Niel, *Antonie van Leeuwenhoek, J. Microbiol. Serol.* **12,** 156–166 (1947).
74. A. Manten, "Phototaxis, Phototropism, and Photosynthesis in Purple Bacteria and Blue-Green Algae," pp. 1–87. Dissertation, Utrecht, Holland, 1948.
75. C. B. van Niel, *Arch. Mikrobiol.* **3,** 1–112 (1931).
76. J. B. Thomas, *Biochim. et Biophys. Acta.* **5,** 186–196 (1950).
77. J. B. Thomas and L. E. Nijenhuis, *Biochim. et Biophys. Acta* **6,** 317–324 (1950).
78. J. M. W. Milatz and A. Manten, *Biochim. et Biophys. Acta* **11,** 17–27 (1953).
79. J. Links, "Een Hypothese over het Mechanisme van de (Phobo-) Chemotaxis," pp. 1–52. Thesis, Leiden, Holland, 1955.
80. P. A. Dangeard, *Ann. protistol. (Paris)* **1,** 3 (1928); cited after Manten, 1948, see reference 74.
80a. A. M. Mayer and A. Poljakoff-Mayber, *Physiol. Plantarum* **12,** 8–14 (1959).
81. S. O. Mast, *J. Exptl. Zool.* **22,** 471–528 (1917).
82. S. O. Mast, *Arch. Protistenk.* **60,** 197–220 (1928).
83. A. Luntz, *Z. vergleich. Physiol.* **14,** 68–92 (1931).
84. H. Laurens and H. D. Hooker, *J. Exptl. Zool.* **30,** 345–368 (1920).
85. F. Oltmanns, *Z. Botan.* **9,** 257–338 (1917).
86. E. Bünning and G. Schneiderhöhn, *Arch. Mikrobiol.* **24,** 80–89 (1956).
87. S. O. Mast, *Z. vergleich. Physiol.* **5,** 730–738 (1927).
87a. J. J. Wolken, *Ann. N. Y. Acad. Sci.* **74,** 165–171 (1958).
88. R. Kuhn, J. Stene, and N. Sörensen, *Ber. deut. chem. Ges.* **72,** 1688–1689 (1939).
88a. J. N. Hartshorne, *New Phytologist* **52,** 292–297 (1953).
88b. I. Gössel, *Arch. Mikrobiol.* **27,** 288–305 (1957).
89. A. Zurzycka and J. Zurzycki, *Acta Soc. Botan. Polon.* **20,** 665–680 (1950).
90. A. Zurzycka, *Acta Soc. Botan. Polon.* **21,** 17–37 (1951).
91. J. Zurzycki and A. Zurzycka, *Acta Soc. Botan. Polon.* **24,** 663–674 (1955).
92. J. Zurzycki, *Acta Soc. Botan. Polon.* **24,** 27–63 (1955).

93. L. N. Babushkin, *Doklady Akad. Nauk. S. S. S. R.* **103,** 333–335, 507-510 (1955); *Chem. Abstr.* **50,** 1959 (1956).
93*a*. W. Haupt, *Naturwissenschaften* **11,** 273–274 (1958).
93*b*. G. Mosebach, *Planta* **52,** 3–46 (1958).
94. H. I. Virgin, *Physiol. Plantarum.* **4,** 255–357 (1951); **7,** 343–353 (1954).
95. V. J. Koningsberger, *Rec. trav. botan. néerl.* **19,** 1–136 (1922).
96. C. Sonne, *Strahlentherapie* **31,** 778–785 (1929).
97. E. S. Castle, *J. Gen. Physiol.* **14,** 701–711 (1931).
98. E. S. Johnston, *Smithsonian Inst. Publs., Misc. Collections* **92,** 1–17 (1934).
99. A. W. Galston and R. S. Baker, *Am. J. Botany* **36,** 773–780 (1949).
100. W. Shropshire, Jr. and R. B. Withrow, *Plant Physiol.* **33,** 360–365 (1958).
101. F. Bachmann and F. Bergmann, *Planta* **10,** 744–755 (1930).
102. S. H. Voerkel, *Planta* **21,** 156–205 (1933).
103. G. Wald and H. G. duBuy, *Science* **84,** 247 (1936).
104. H. H. Inhoffen, F. Bohlmann, and G. Rummert, *Ann.* **571,** 75–83 (1950).
105. L. Zechmeister, *Chem. Revs.* **34,** 267–344 (1944).
106. G. Wald, *in* "Modern Problems in Ophthalmology" (E. B. Streiff and J. Babel, eds.), Vol. 1, pp. 173–188. Karger, Basel, 1957; and earlier papers cited therein.
107. M. Nishimura and K. Takamatsu, *Nature* **180,** 699–700 (1957).
108. E. Bünning, *Planta* **26,** 719–736 (1937); **27,** 148–158 (1937).
109. K. Shibata, A. A. Benson, and M. Calvin, *Biochim. et Biophys. Acta* **15,** 461–470 (1954).
110. P. Karrer, H. Salomon, K. Schöpp, E. Schlittler, and H. Fritzsche, *Helv. Chim. Acta* **17,** 1010–1013 (1934).
111. R. H. Goodwin, *Ann. Rev. Plant Physiol.* **4,** 283–304 (1953).
112. T. W. Goodwin and W. Lijinsky, cited by T. W. Goodwin in *Botan. Rev.* **18,** 291–316 (1952).
113. J. Reinert, *Naturwissenschaften* **39,** 47–48 (1952).
114. R. S. Bandurski, A. W. Galston, R. S. Baker, and R. S. Roberston, unpublished experiments, 1950; cf. also reference 23.
115. H. I. Virgin, *Physiol. Plantarum* **10,** 170–186 (1957).
116. G. Turian, *Helv. Chim. Acta* **33,** 1988–1993 (1950).
117. N. T. Clare, *in* "Radiation Biology" (A. Hollaender, ed.), Vol. 3, pp. 693–723. McGraw-Hill, New York, 1956.
118. M. Griffiths, W. R. Sistrom, G. Cohen-Bazire, and R. Y. Stanier, *Nature* **176,** 1211–1215 (1955).
119. G. J. Schuringa, "De Foto-inactiveering van Auxine-a-lacton." Dissertation, Utrecht, 1941.
120. F. Kögl and G. J. Schuringa, *Z. physiol. Chem., Hoppe-Seyler's* **280,** 148–161 (1944).
121. J. Reinert, *Z. Botan.* **41,** 103–122 (1953).
122. L. Brauner, *Z. Botan.* **41,** 291–341 (1953).
123. A. W. Galston, *Proc. Natl. Acad. Sci. U. S.* **35,** 10–17 (1949).
124. A. W. Galston and R. S. Baker, *Science* **109,** 485–486 (1949).
124*a*. H. A. Harbury, K. F. La Noue, P. A. Loach, and R. M. Amick, *Proc. Natl. Acad. Sci. (U. S.)* **45,** 1708–1717 (1959).
124*b*. H. Theorell and Å. Åkeson, *Arch. Biochem. Biophys.* **65,** 439–448 (1956).
124*c*. N. A. Frigerio and H. A. Harbury, *J. Biol. Chem.* **231,** 135–157 (1958).
125. F. Skoog, *J. Cellular Comp. Physiol.* **7,** 227–270 (1935).

126. M. G. Ferri, *Arch. Biochem. Biophys.* **31,** 127–131 (1951).
127. C. L. Mer, *Plant Physiol.* **32,** 175–185 (1957).
128. G. Wald, *Vitamins and Hormones* **1,** 195–227 (1945).
129. L. Brauner, *Z. Botan.* **43,** 467–498 (1955).
130. E. Bünning, I. Dorn, G. Schneiderhohn, and I. Thorning, *Ber. deut. botan. Ges.* **66,** 333–340 (1953).
131. E. Bünning, *Z. Botan.* **43,** 167–174 (1955).
132. C. S. French and V. M. K. Young, *in* "Radiation Biology" (A. Hollaender, ed.), Vol. 3, pp. 343–391. McGraw-Hill, New York, 1956.
133. H. F. Blum, *in* "Radiation Biology" (A. Hollaender, ed.), Vol. 2, pp. 487–528. McGraw-Hill, New York, 1955.
134. M. J. Carlile, *Nature* **180,** 202 (1957).
135. I. Thorning, *Z. Botan.* **43,** 175–179 (1955).
135a. M. T. J. Abbott and J. F. Grove, *Exptl. Cell Research* **17,** 95–104 (1959).
135b. G. M. Curry and H. E. Gruen, *Proc. Natl. Acad. Sci.* **45,** 797–804 (1959).
136. R. Cohen and M. Delbrück, *Abstr. Biophys. Soc.* p. 37, February 5–7 meetings (1958).
137. H. Burckhardt, *Z. Botan.* **18,** 273–336 (1926).
138. A. W. Galston, J. Bonner, and R. S. Baker, *Arch. Biochem. Biophys.* **42,** 456–470 (1953).
139. A. W. Galston and R. S. Baker, *Am. J. Botany* **38,** 190–195 (1951).
140. R. H. Kenten, *Biochem. J.* **59,** 110–121 (1955).
141. W. A. Andreae, *Arch. Biochem. Biophys.* **55,** 584–586 (1955).
142. E. R. Waygood, A. Oaks, and G. A. Maclachlan, *Can. J. Botany* **34,** 54–59. 905–926 (1956).
143. E. R. Waygood and G. A. Maclachlan, *Chem. in Can.* **8,** 40–45 (1956).
144. F. G. Gustafson, *Plant Physiol.* **23,** 373–378 (1948).
145. F. G. Gustafson, *Plant Physiol.* **30,** 444–447 (1955).
146. P. M. Ray and K. V. Thimann, *Arch. Biochem. Biophys.* **64,** 175–192 (1956).
147. L. Sequeira and T. A. Steeves, *Plant Physiol.* **29,** 11–16 (1954).
147a. P. M. Ray, *Ann. Rev. Plant. Physiol.* **9,** 81–118 (1958).
147b. H. von Guttenberg, *Planta* **53,** 412–433 (1959).
148. J. Meyer and E. Pohl, *Naturwissenschaften* **43,** 114–115 (1956).
149. J. Meyer, *Z. Botan.* **46,** 125–160 (1958).
149a. S. C. Fang P. Theisen, and J. S. Butts, *Plant Physiol.* **34,** 26–32 (1959).
150. S. A. Gordon, *Quart. Revs. Biol.* **32,** 3–14 (1957).
151. A. Fisher, *Planta* **43,** 288–314 (1954).
152. A. R. Schrank, *Symposia Soc. Exptl. Biol.* **11,** 95–117 (1957).
153. H. P. Bottelier, *Rec. trav. botan. néerl.* **31,** 474–582 (1934).
154. K. V. Thimann and B. M. Sweeney, *J. Gen. Physiol.* **21,** 123–135 (1937); **21,** 439–461 (1938); **25,** 841–854 (1942).
155. J. M. Kelso and J. S. Turner, *Australian J. Biol. Sci.* **8,** 19–35 (1955).
156. G. Raydt, *Jahrb. wiss. Botan.* **64,** 731–769 (1925).
157. L. Brauner and Y. Vardar, *Rev. fac. sci. univ. Istanbul* **B15,** 269–299 (1950).
158. H. C. Yin, *Am. J. Botany* **28,** 250–261 (1941).
159. F. Laibach and O. Fischnich, *Biol. Zentr.* **56,** 62–68 (1936).
160. F. W. Went, *Biol. Zentr.* **56,** 449–463 (1936).
161. A. R. Schrank, *J. Cellular Comp. Physiol.* **32,** 143–159 (1948).
162. L. R. Koller, *in* "Measurement of Radiant Energy" (W. E. Forsythe, ed.), pp. 216–245. McGraw-Hill, New York, 1937.

163. A. R. Schrank, *Ann. Rev. Plant. Physiol.* **1,** 59–74 (1950).
164. A. N. J. Heyn, *Rec. trav. botan. néerl.* **28,** 113–244 (1931); *Jahrb. wiss. Botan.* **79,** 753–799 (1934).
165. H. Söding, *Jahrb. wiss. Botan.* **74,** 127–151 (1931); *Ber. deut. botan. Ges.* **50,** 117–123 (1933).
166. T. Tagawa and J. Bonner, *Plant Physiol.* **32,** 207–212 (1957).

61. P. Boysen-Jensen, *Planta* **5,** 464–477 (1928).
62. H. G. duBuy and E. L. Nuernbergk, *Koninkl. Ned. Akad. Wetenschap., Proc.* **33,** Pt. 1, 542–556 (1930).
63. H. Söding, "Die Wuchsstofflehre." Thieme, Stuttgart, 1952.
64. E. Bünning, *Planta* **27,** 583–610 (1937).
65. K. V. Thimann and J. Bonner, *Proc. Natl. Acad. Sci. U. S.* **18,** 692–701 (1932).
66. H. J. Reisener, *Z. Botan.* **46,** 474–505 (1958).
66a. A. W. Galston, *Botan. Rev.* **16,** 361–378 (1950).
67. P. R. Burkholder and E. S. Johnston, *Smithsonian Inst. Publs., Misc. Collections* **95,** 1–14 (1937).
68. C. Brown, *in* "The Physiology of Forest Trees" (K. V. Thimann, ed.), pp. 511–525. Ronald Press, New York, 1958.
69. E. Bünning, H. J. Reisener, F. Weygand, H. Simon, and J. F. Klebe, *Z. Naturforsch.* **11b,** 363–364 (1956).
70. S. A. Gordon and M. Eib, *Plant Physiol.* **31,** Suppl. xiv, Proc. Am. Soc. Plant Physiol. (1956).
71. L. Brauner, *Symposia Soc. Exptl. Biol.* **11,** 86–94 (1957).
72. B. B. Stowe and T. Yamaki, *Ann. Rev. Plant. Physiol.* **8,** 181–216 (1957).
73. C. B. van Niel, *Antonie van Leeuwenhoek, J. Microbiol. Serol.* **12,** 156–166 (1947).
74. A. Manten, "Phototaxis, Phototropism, and Photosynthesis in Purple Bacteria and Blue-Green Algae," pp. 1–87. Dissertation, Utrecht, Holland, 1948.
75. C. B. van Niel, *Arch. Mikrobiol.* **3,** 1–112 (1931).
76. J. B. Thomas, *Biochim. et Biophys. Acta.* **5,** 186–196 (1950).
77. J. B. Thomas and L. E. Nijenhuis, *Biochim. et Biophys. Acta* **6,** 317–324 (1950).
78. J. M. W. Milatz and A. Manten, *Biochim. et Biophys. Acta* **11,** 17–27 (1953).
79. J. Links, "Een Hypothese over het Mechanisme van de (Phobo-) Chemotaxis," pp. 1–52. Thesis, Leiden, Holland, 1955.
80. P. A. Dangeard, *Ann. protistol. (Paris)* **1,** 3 (1928); cited after Manten, 1948, see reference 74.
80a. A. M. Mayer and A. Poljakoff-Mayber, *Physiol. Plantarum* **12,** 8–14 (1959).
81. S. O. Mast, *J. Exptl. Zool.* **22,** 471–528 (1917).
82. S. O. Mast, *Arch. Protistenk.* **60,** 197–220 (1928).
83. A. Luntz, *Z. vergleich. Physiol.* **14,** 68–92 (1931).
84. H. Laurens and H. D. Hooker, *J. Exptl. Zool.* **30,** 345–368 (1920).
85. F. Oltmanns, *Z. Botan.* **9,** 257–338 (1917).
86. E. Bünning and G. Schneiderhöhn, *Arch. Mikrobiol.* **24,** 80–89 (1956).
87. S. O. Mast, *Z. vergleich. Physiol.* **5,** 730–738 (1927).
87a. J. J. Wolken, *Ann. N. Y. Acad. Sci.* **74,** 165–171 (1958).
88. R. Kuhn, J. Stene, and N. Sörensen, *Ber. deut. chem. Ges.* **72,** 1688–1689 (1939).
88a. J. N. Hartshorne, *New Phytologist* **52,** 292–297 (1953).
88b. I. Gössel, *Arch. Mikrobiol.* **27,** 288–305 (1957).
89. A. Zurzycka and J. Zurzycki, *Acta Soc. Botan. Polon.* **20,** 665–680 (1950).
90. A. Zurzycka, *Acta Soc. Botan. Polon.* **21,** 17–37 (1951).
91. J. Zurzycki and A. Zurzycka, *Acta Soc. Botan. Polon.* **24,** 663–674 (1955).
92. J. Zurzycki, *Acta Soc. Botan. Polon.* **24,** 27–63 (1955).

93. L. N. Babushkin, *Doklady Akad. Nauk. S. S. S. R.* **103,** 333–335, 507-510 (1955); *Chem. Abstr.* **50,** 1959 (1956).
93*a*. W. Haupt, *Naturwissenschaften* **11,** 273–274 (1958).
93*b*. G. Mosebach, *Planta* **52,** 3–46 (1958).
94. H. I. Virgin, *Physiol. Plantarum.* **4,** 255–357 (1951); **7,** 343–353 (1954).
95. V. J. Koningsberger, *Rec. trav. botan. néerl.* **19,** 1–136 (1922).
96. C. Sonne, *Strahlentherapie* **31,** 778–785 (1929).
97. E. S. Castle, *J. Gen. Physiol.* **14,** 701–711 (1931).
98. E. S. Johnston, *Smithsonian Inst. Publs., Misc. Collections* **92,** 1–17 (1934).
99. A. W. Galston and R. S. Baker, *Am. J. Botany* **36,** 773–780 (1949).
100. W. Shropshire, Jr. and R. B. Withrow, *Plant Physiol.* **33,** 360–365 (1958).
101. F. Bachmann and F. Bergmann, *Planta* **10,** 744–755 (1930).
102. S. H. Voerkel, *Planta* **21,** 156–205 (1933).
103. G. Wald and H. G. duBuy, *Science* **84,** 247 (1936).
104. H. H. Inhoffen, F. Bohlmann, and G. Rummert, *Ann.* **571,** 75–83 (1950).
105. L. Zechmeister, *Chem. Revs.* **34,** 267–344 (1944).
106. G. Wald, *in* "Modern Problems in Ophthalmology" (E. B. Streiff and J. Babel, eds.), Vol. 1, pp. 173–188. Karger, Basel, 1957; and earlier papers cited therein.
107. M. Nishimura and K. Takamatsu, *Nature* **180,** 699–700 (1957).
108. E. Bünning, *Planta* **26,** 719–736 (1937); **27,** 148–158 (1937).
109. K. Shibata, A. A. Benson, and M. Calvin, *Biochim. et Biophys. Acta* **15,** 461–470 (1954).
110. P. Karrer, H. Salomon, K. Schöpp, E. Schlittler, and H. Fritzsche, *Helv. Chim. Acta* **17,** 1010–1013 (1934).
111. R. H. Goodwin, *Ann. Rev. Plant Physiol.* **4,** 283–304 (1953).
112. T. W. Goodwin and W. Lijinsky, cited by T. W. Goodwin in *Botan. Rev.* **18,** 291–316 (1952).
113. J. Reinert, *Naturwissenschaften* **39,** 47–48 (1952).
114. R. S. Bandurski, A. W. Galston, R. S. Baker, and R. S. Roberston, unpublished experiments, 1950; cf. also reference 23.
115. H. I. Virgin, *Physiol. Plantarum* **10,** 170–186 (1957).
116. G. Turian, *Helv. Chim. Acta* **33,** 1988–1993 (1950).
117. N. T. Clare, *in* "Radiation Biology" (A. Hollaender, ed.), Vol. 3, pp. 693–723. McGraw-Hill, New York, 1956.
118. M. Griffiths, W. R. Sistrom, G. Cohen-Bazire, and R. Y. Stanier, *Nature* **176,** 1211–1215 (1955).
119. G. J. Schuringa, "De Foto-inactiveering van Auxine-a-lacton." Dissertation, Utrecht, 1941.
120. F. Kögl and G. J. Schuringa, *Z. physiol. Chem., Hoppe-Seyler's* **280,** 148–161 (1944).
121. J. Reinert, *Z. Botan.* **41,** 103–122 (1953).
122. L. Brauner, *Z. Botan.* **41,** 291–341 (1953).
123. A. W. Galston, *Proc. Natl. Acad. Sci. U. S.* **35,** 10–17 (1949).
124. A. W. Galston and R. S. Baker, *Science* **109,** 485–486 (1949).
124*a*. H. A. Harbury, K. F. La Noue, P. A. Loach, and R. M. Amick, *Proc. Natl. Acad. Sci. (U. S.)* **45,** 1708–1717 (1959).
124*b*. H. Theorell and Å. Åkeson, *Arch. Biochem. Biophys.* **65,** 439–448 (1956).
124*c*. N. A. Frigerio and H. A. Harbury, *J. Biol. Chem.* **231,** 135–157 (1958).
125. F. Skoog, *J. Cellular Comp. Physiol.* **7,** 227–270 (1935).

tion to which animals and plants are sensitive. The spectrum of electromagnetic radiation covers the enormous range of about 10^{16} in wavelength, from gamma rays 10^{-10} cm. long to radio waves several kilometers long. Within this vast domain, the radiations that stimulate phototropism and vision include the almost negligible range of about 1 to 2 (about 350–700 mμ). Why just this range of wavelengths? The answer is that these responses of living organisms are adapted to sunlight. The energy of sunlight as it reaches the surface of the earth presents a broad maximum centering at about 500 mμ (*1*); and clustered under this as under an umbrella one finds all the action spectra of phototropism and vision in plants and animals (Fig. 1).

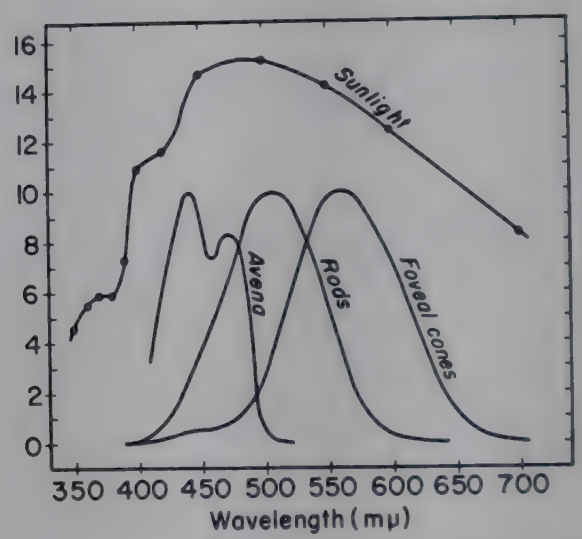


FIG. 1. Energy of sunlight as it reaches the earth's surface, compared with the spectral sensitivities of various organisms. The sunlight spectrum shows the relative energies delivered in 10 mμ intervals as measured by Pettit (*1*) (mean noonday sunlight for midsummer in Tucson, Arizona). Below this are shown the spectral sensitivity for the bending reaction of the oat shoot, *Avena* (Johnston, *2*); and measurements of the spectral sensitivities of human rod vision and foveal cone vision (Wald, *3*).

A second important recognition is that throughout their entire range these excitations appear to be derived chemically from a single, closely knit family of compounds, the carotenoids. This relationship persists from phototropism in molds to vision in man (*4*, *5*, *6*).

The carotenoids constitute a group of yellow to red, fat-soluble molecules, which owe their color to hydrocarbon chains possessing alternate single and double bonds, forming so-called conjugated systems. The carotenoids proper possess 40 carbon atoms in the molecule; their most prevalent and familiar representatives are β-carotene, $C_{40}H_{56}$, and leaf xanthophyll, $C_{40}H_{54}(OH)_2$. In plants and some lower invertebrates, these are the substances that appear to govern photoreception; but with the emergence of well-formed eyes in cephalopods,

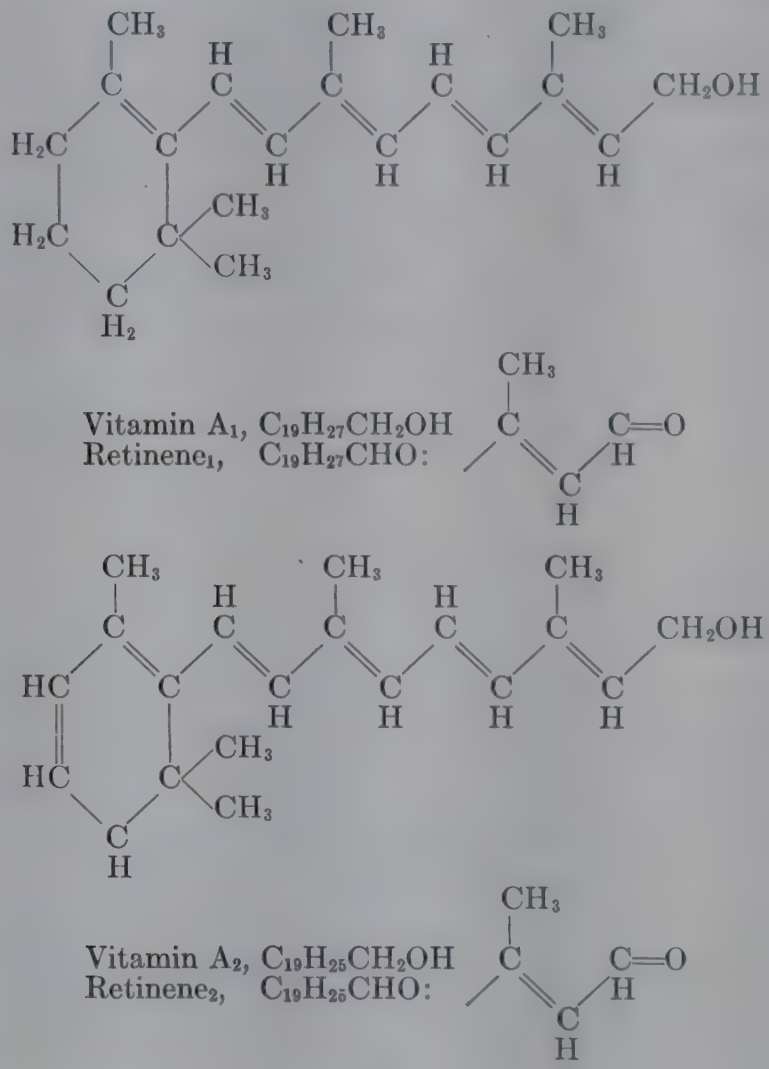
arthropods, and vertebrates, we find that the primary visual functions have passed to a group of carotenoid derivatives—*haplocarotenoids*—having 20 carbon atoms in the molecule. Furthermore, unlike the plant carotenoids which are synthesized *de novo*, these molecules or the plant carotenoids from which they are made must be supplied in the diet. They therefore have the position in the animal economy of vitamins, the vitamins A. So far as we know, all the photosensitive pigments involved in animal vision are formed by joining aldehydes of the vitamins A—the so-called retinenes—to proteins. Even here, however, one finds the larger plant carotenoids and some of their more highly oxidized derivatives—such as astaxanthin, $C_{40}H_{52}O_4$—in the role of accessory pigments, concerned with filtering and absorbing the light which strikes the visual receptors. So it is that the filter pigments of the cones of many day birds and turtles are carotenoids (*7*) and the pigment of the yellow patch (*macula lutea*) of the human retina is also a carotenoid (*3*).

What properties particularly fit the carotenoids and vitamins A for photoreception? I think in the first instance the fact that they are pigments and readily form further pigments by association with proteins. Second, these pigments tend to absorb light maximally in the range of wavelengths in which the energy of sunlight reaching the surface of the earth is most concentrated, a point already considered in Fig. 1. In this the carotenoids and their derivatives are not unique; the phycobilins would have done just as well. A third property is beginning to emerge as of the utmost importance. Unlike other common natural pigments—porphyrins, phycobilins, anthocyanins—the color of which is associated with conjugated systems bound more or less rigidly in *rings*, the carotenoids and vitamins A have long straight-chain conjugated systems, which readily undergo *cis-trans* or geometrical isomerization (*8*). That is, these molecules can undergo a variety of drastic changes in *shape*, involving only changes in geometrical configuration about their double bonds. These changes are readily induced by simple absorption of light. The capacity of a carotenoid to interact with a protein depends intimately upon its shape; a change in shape can modify or abolish such interaction; and both the interaction and its cessation are readily invoked by light. This is the fundamental basis of the formation and bleaching of the visual pigments (*9, 10*); and may be the key to the special suitability of carotenoids in all types of photoreception.

II. Visual Systems of Vertebrates

The biochemistry of vertebrate vision has at present reached the following simple position (*11*). Two vitamins A are known—A_1 and A_2

—and their corresponding aldehydes, retinene$_1$ and retinene$_2$, which differ only in the possession by the latter of one added double bond in the ring (I).



(I)

There are also two families of proteins, called opsins, one found in rods (scotopsins), the other in cones (photopsins). Combinations of the two retinenes with the two opsins yield the four known pigments of vertebrate vision as shown.

Retinene	Opsin	Photosensitive Pigment	λ_{max} (mμ)
Retinene$_1$ +	Rod opsin	→ Rhodopsin	500
Retinene$_2$ +	Rod opsin	→ Porphyropsin	522
Retinene$_1$ +	Cone opsin	→ Iodopsin	562
Retinene$_2$ +	Cone opsin	→ Cyanopsin	620

The λ_{max} cited here for rhodopsin or porphyropsin is a prevalent value; λ_{max} may differ considerably from this in individual instances, of which more later. The λ_{max} cited for iodopsin is that of the chicken and

pigeon, which are best characterized; that of cyanopsin is for the synthetic product (*12*); it lies close to the maximum spectral sensitivity of cone vision in the Greek tortoise and the tench, as measured by Granit (*13*).

In all these systems the retinenes and vitamins A are interconverted by the enzyme, alcohol dehydrogenase, acting together with coenzyme I (DPN, cozymase) according to Eqs. 1 and 2 (*14*).

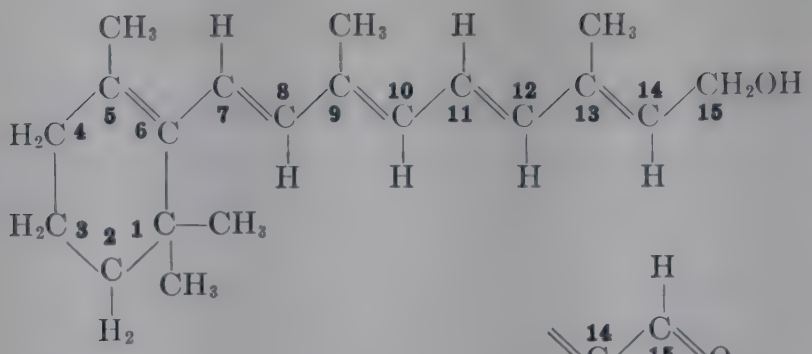
$$\underset{\text{Vitamin } A_1}{C_{19}H_{27}CH_2OH} + DPN^+ \xrightarrow{\text{Alcohol dehydrogenase}} \underset{\text{Retinene}_1}{C_{19}H_{27}CHO} + DPNH + H^+ \quad (1)$$

$$\underset{\text{Vitamin } A_2}{C_{19}H_{25}CH_2OH} + DPN^+ \xrightarrow{\text{Alcohol dehydrogenase}} \underset{\text{Retinene}_2}{C_{19}H_{25}CHO} + DPNH + H^+ \quad (2)$$

In both these oxido-reductions, the point of equilibrium lies far over toward the side of reduction, toward the production of vitamins A. When the visual pigments are bleached in the retina by light, the retinene which results is very quickly reduced almost entirely to the corresponding vitamin A. Its reoxidation to retinene requires a special device. Retinene combines with opsin spontaneously to yield a visual pigment; in the dark this reaction goes virtually to completion. Opsin is in effect a physiological retinene-trapping reagent, which in the dark, by removing retinene as fast as formed, forces the continuous oxidation of more vitamin A to retinene. The synthesis of visual pigments proceeds therefore as a coupled reaction, in which the combination of retinene with opsin drives the oxidation of vitamin A. For this reason it is also self-limiting; when all the opsin has been converted to visual pigment, no further vitamin A is oxidized (*15*).

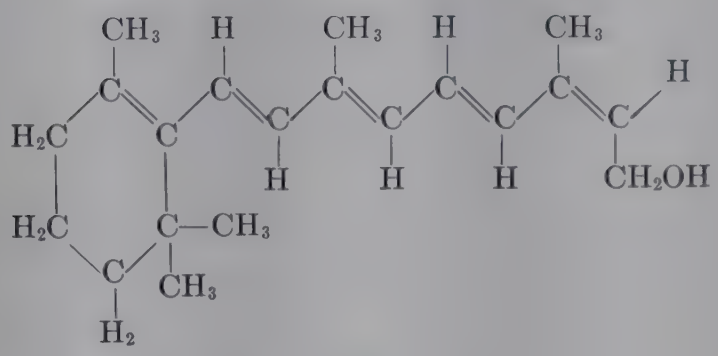
An important point remains to be considered. It is not enough to have vitamin A_1 or A_2 to make a visual pigment; these substances must have the right *shape* (*9*). Like other substances possessing multiple double bonds, vitamin A and retinene occur in a variety of shapes, *cis-trans* or geometrical isomers of one another. The most common and stable of these configurations is all-*trans* (Fig. 2). This is the usual form of crystalline and synthetic vitamin A; but the synthesis of visual pigments requires instead one of the *cis* isomers, called neo-b.

Of the four double bonds in the side chain of vitamin A_1 or A_2, it was expected originally that only two should go readily into the *cis* configuration to yield stable products: those in positions 9 and 13 (Fig. 2). The conversion of a *trans* to a *cis* linkage ordinarily involves a rotation at the double bond through 180°, and produces a bend in the molecule. At positions 7 and 11, however, this rotation cannot be completed, because of steric hindrance: side groups run into each other, either methyl groups at double bond 7, or a methyl and a hydrogen at double bond 11.

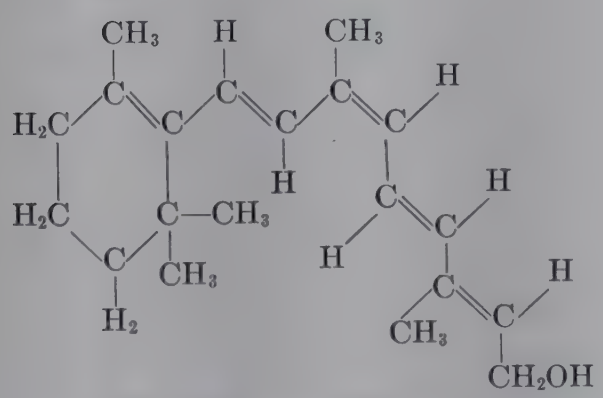


all-*trans* Vitamin A

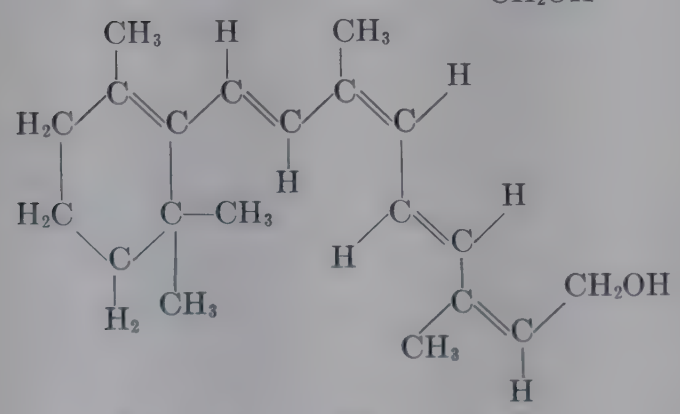
Retinene



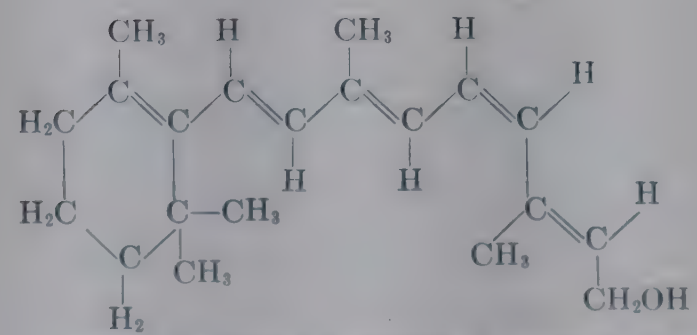
13-*cis* (*neo*-a)



9-*cis* (*iso*-a)



9,13-*dicis* (*iso*-b)



11-*cis* (*neo*-b)

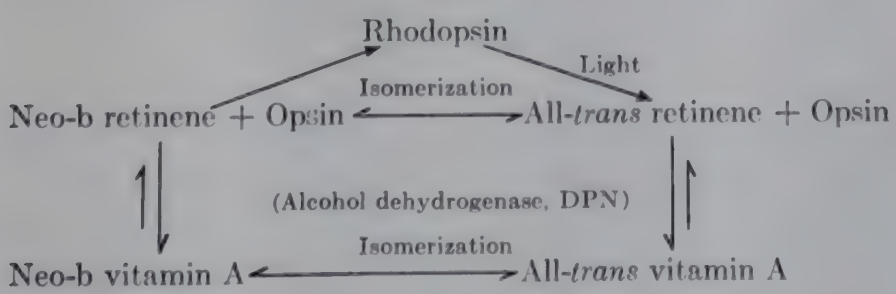
FIG. 2. Geometric isomers of vitamin A and retinene. A similar series of structures, differing only by possessing an extra double bond in the 3,4 position, represents vitamin A_2 and retinene$_2$. The upper four structures represent the unhindered, and hence most probable configurations. The lowermost structure is the hindered *cis* isomer from which all known visual pigments are formed.

At such *hindered cis* linkages, therefore, the molecule should be not only bent but twisted; and since planarity is the condition for free resonance, and resonance promotes stability, such hindered *cis* linkages were expected to be both rare and unstable.

It is now known however that the neo-b isomer from which all known visual pigments are formed possesses such a hindered *cis* linkage in the 11 position (*16*). The much greater hindrance at double bond 7 has so far prevented the synthesis of the 7-*cis* molecule; but the 11-*cis*, neo-b isomer, given the necessary energy, forms readily, and once formed is surprisingly stable. Indeed, under proper conditions it is a highly favored isomer. One can isomerize the retinenes by simple exposure to light, provided it contains the blue, violet, and near ultraviolet components that the retinenes absorb. Any isomer of retinene, on irradiation with such light, goes over into a steady state mixture of all possible isomers. In a homopolar solvent such as hexane, this mixture is about 95% all-*trans*; but in such a polar solvent as ethyl alcohol, the *cis* isomers are much more prevalent, and about 25% of the steady state mixture is in the hindered, 11-*cis* configuration (*9*, *17*).

All the visual pigments now known appear to have as prosthetic group the same neo-b, 11-*cis*, configuration of retinene. This is true equally of the rod and cone pigments of vertebrates, whether made of retinene$_1$ or retinene$_2$; and of the visual pigment of the squid, the only invertebrate pigment in which the prosthetic group has been identified.

With this generalization one can couple another. The bleaching of visual pigments yields almost entirely the all-*trans* isomer of retinene as the initial product. That is, a retinene enters the synthesis of visual pigment in one configuration, and emerges in another. It enters as the bent and twisted 11-*cis* isomer, and comes out relatively straight. Then it must be bent and twisted back into the neo-b configuration before it can take part again in the synthesis of visual pigment. A cycle of geometric isomerization is therefore an intrinsic part of all the visual systems we know. In the rhodopsin system this takes the form (*9*) shown (II). A similar diagram can be given for each of the other visual



(II)

systems (*11*).

Far from being adventitious, these changes of geometric confi
tion lie at the heart of the bleaching and resynthesis of the visual
ments. Recent experiments by Hubbard and Kropf (10) show that
light does to a visual pigment, and all that it does, is to isomerize
neo-b chromophore to all-trans. The neo-b isomer fits a site on
surface of the opsin so intimately as to permit a high degree of
action, which by promoting the resonance forms of retinene, dec
the pigmentation (Fig. 5). The absorption of a quantum of

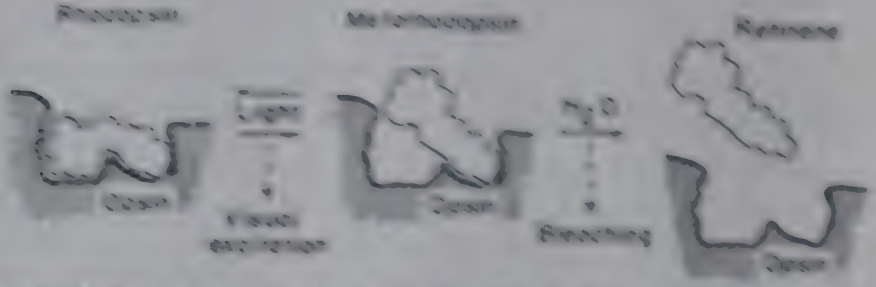


FIG. 5. Hypothetical scheme showing the effect of light on vertebrate rhod
to rhodopsin, the neo-b (11-cis) chromophore fits into the chromophore s
opsin. This site is fitted also, though less well, by the neo-a (9-cis) chrom
(dotted line) of isorhodopsin. Light isomerizes the 11 chromophore to the all
configuration, thus decreasing the interaction between the chromophore and
The result is all-trans metarhodopsin, which hydrolyzes readily to retinene
opsin. The isomerization of rhodopsin to metarhodopsin is probably responsi
visual excitation, but bleaching is due to the hydrolysis of metarhodopsin.
(Hubbard and Kropf, 10.)

straightens out the chromophore to the all-trans configuration, whi
longer fits the site, so resulting in loss of interaction and consequen
of color, i.e., bleaching. The reisomerization of the all-trans isom
neo-b makes it fit opsin again, permitting the regeneration of the p
sensitive pigment.

The universal choice of a peculiar, hindered cis isomer to mak
visual pigments probably has a related explanation. Just becau
its inherent improbability, this isomer on absorbing light goes over
quickly and completely to the all-trans configuration. The visual
ments therefore achieve the highest sensitivity to light—the hi
quantum efficiency of bleaching—by having this configurati
chromophore.

This generality of chromophore is coupled with a wide ra
species specificity of opsins. So far as we know, every species tha
has at least one specific opsin, different from that found in every
species. In this the opsins are like proteins generally. In hemoglobi
example, a single prosthetic group, ferroheme, is bound to an enor
variety of different globins, each species having at least one and
times several globins different from those of all other species.

the specific differences in the opsins confer distinctive properties upon and upon the visual pigments derived from them. These properties ably beginning to be explored, but already we can recognize small large specific differences in absorption spectrum, in the rate of tration of opsin with neo-b retinene (18), in the temperature range ergy of activation for denaturation (19), and in a few instances erences in isoelectric point (frog, 4.53 (20); cattle, about 5.4) (21). times for the synthesis of rhodopsin in solution are already known ry with species from 16–20 seconds (alligator, carp) to 4–5 minutes , cattle, chicken) (22*) (18). The chicken cone pigment, iodopsin, nthesized in solution more than 500 times as fast as chicken opsin (22); but the rhodopsins of the butterfish and cock are syn- ed as rapidly as chicken iodopsin (23). We find not only a wide of rates of synthesis of rhodopsin, but at the upper extremes they ap with the generally more rapid rates of synthesis of iodopsin.

comparable situation involving absorption spectra has lately caused otward account of concern in some quarters. In part the reason is that while their chemistry was still obscure the visual pigments to be known and classified on the basis of their color. Their names color names: rhodopsin means rose-colored eye pigment; porphy- n, purple; iodopsin, violet; cyanopsin, blue eye pigment. From the ning it was recognized that the absorption spectra vary somewhat. or a long time all the known rhodopsins could be characterized as g λ_{max} between the limits 500 ± 2 mμ, and porphyropsins ± 2 mμ. Lately these limits have expanded, as inevitably they must e size of sample is increased. Human rhodopsin lies at 491 mμ (24), rhodopsin at 524 mμ (25), quite apart from the discovery of new al groupings of rhodopsins, as in deep-sea fishes (λ_{max} about 490 (26) and invertebrates (octopus, 475 mμ (27); euphausiids, 493 (28). Certain freshwater fishes may have porphyropsins with λ_{max} mμ (32, 33), and the iodopsins of certain marine fishes, rather than at 562 mμ, seem to be at 530–550 mμ (29).

Most of the known rhodopsins of amphibia, reptiles, mammals, and ne forms of marine fish, however, center close to 500 mμ, and it is able that the great bulk of such rhodopsins have λ_{max} lying between imits 495 and 502 mμ. This is now an old story, and grows tedious. rhodopsins with highly divergent λ_{max} now seem worth writing rs about, so that from now on the literature will abound in aberrant ra. One should not be misled by this into a distorted view of the tion as a whole. Nor is it necessary to be confused by the observa- that gecko rhodopsin lies at as long a wavelength as the usual por- opsin, or that the rhodopsins of deep-sea fishes look orange instead se-colored. We can retain the old color names which are traditional

and familiar, but need now to adopt definitions based on the known chemistry. From this point of view we shall define a rhodopsin as a visual pigment that contains neo-b retinene$_1$ bound to rod opsin, and porphyropsin as a pigment containing neo-b retinene$_2$ bound to the same class of protein. Since this composition involves a protein, specific differences are expected; they are an important and essential complication of the subject, and should not be the source of either elation or confusion.

What the specific differences in opsins imply chemically is an interesting question, the details of which still need to be explored. In such a molecule the prosthetic group is bound at a particular site, involving directly a small constellation of amino acids, ordinarily a very small fragment of the whole protein. This fragment probably maintains a measure of constancy throughout one entire family of proteins. Thus it is probable that a specific constellation of amino acids characterizes the rod opsins, and another constellation the cone opsins. Apart from the binding site, all the remainder of the protein can vary endlessly, as one goes from one species to another. Differences in composition in the neighborhood of the binding site probably are the source of specific differences in the properties and behavior of the chromophore; differences in composition that lie more remote can still affect such general properties as isoelectric point, solubility, and molecular weight. The final searching out of specific differences may call for such delicate procedures as the immunological production of specific antibodies, themselves proteins; though present methods of amino acid analysis and the determination of amino acid sequences have begun to make it possible to approach this problem directly.

III. Habitat Relations: Freshwater Fishes versus Marine Fishes and Land Vertebrates

The rods of freshwater teleosts contain porphyropsin, which bleaches to form successively retinene$_2$ and vitamin A$_2$ (*30*, *31*) (Fig. 4). Only about a dozen forms have yet been reported in the literature; but they are widely distributed, and as yet include no exception to this condition. In almost all these animals the λ_{max} of porphyropsin lies at 520–526 mμ (i.e., 523 ± 3 mμ) (*31*). In several instances Dartnall has reported longer λ_{max}: 533 mμ in the tench, pike (*32*), and bleak (*33*). There is nothing intrinsically unlikely in finding porphyropsins at these wavelengths; yet these values are derived by the manipulation of quite impure preparations; and must be coupled with conflicting reports that tench porphyropsin lies at about 520 mμ (*34*) and pike porphyropsin at 525 mμ (*35*).*

* *Note added in proof:* Crescitelli (*35a*) has confirmed 533 mμ as the λ_{max} of tench porphyropsin.

The African lungfish, *Protopterus*, also has only vitamin A_2 in its retina, and may be presumed therefore to possess porphyropsin (*4, 5*).

Most freshwater fishes have also predominantly or exclusively vitamin A_2 in the liver. This however is not always the case. The carp is a notable example of a freshwater fish which, though possessing exclusively vitamin A_2 and porphyropsin in its retina, has predominantly vitamin A_1 in its liver (*30*).

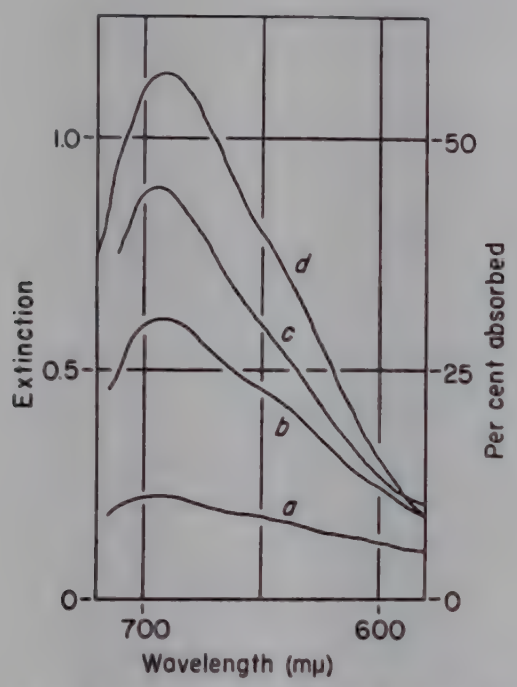


FIG. 4. The vitamin A_2 of the retinas of freshwater fishes. Antimony chloride tests with extracts of wholly bleached retinas display only the absorption band maximal at 690–696 mμ characteristic of vitamin A_2. This result has been obtained invariably in about a dozen widely distributed species of freshwater teleosts. In almost all cases the visual pigment has also been extracted, and found to be porphyropsin, with λ_{max} about 522 mμ. KEY: (*a*) pickerel; (*b*) calico bass; (*c*) white perch; (*d*) carp. (From Wald, *4, 5, 30.*)

In sharp distinction to the freshwater fishes, the rods of marine fishes characteristically contain vitamin A_1 and retinene$_1$, and rhodopsin as visual pigment (*30, 36*) (Fig. 5). Among surface forms, about 20 widely distributed species have been examined, including the smooth and spiny dogfishes as well as bony fishes, all of which present this condition, and in almost all of which the λ_{max} of rhodopsin in solution lies at 500 $\pm$ 2–3 mμ. (One should add to this list, though the supporting data are not yet published, the statement of Kampa (*37*) that she found 39 species of marine fishes from Bermudan waters, representing 15 different families, to contain rhodopsin, retinene$_1$, and vitamin A_1.)

The only exceptions yet found to this condition involve members of the family Labridae, the wrasse-fishes (cunner and tautog), in which the retinas were found some time ago to contain mixtures of vitamins A_1 and A_2, with a great predominance (more than 85%) of A_2 (*30, 43*).

Kampa has since reported finding porphyropsin, retinene$_2$, and vitamin A_2 in two species of the family Coridae, caught near Bermuda (37); and goes on to say, "The position of this family is in considerable doubt, and certain taxonomists feel that it should be included in the family Labridae." Recently Munz (38) has reported finding a Pacific coast labrid, the sheepshead, possessing a rhodopsin (i.e., a vitamin A_1 pigment) in its retina with λ_{max} 497 mμ, accompanied by a small amount of

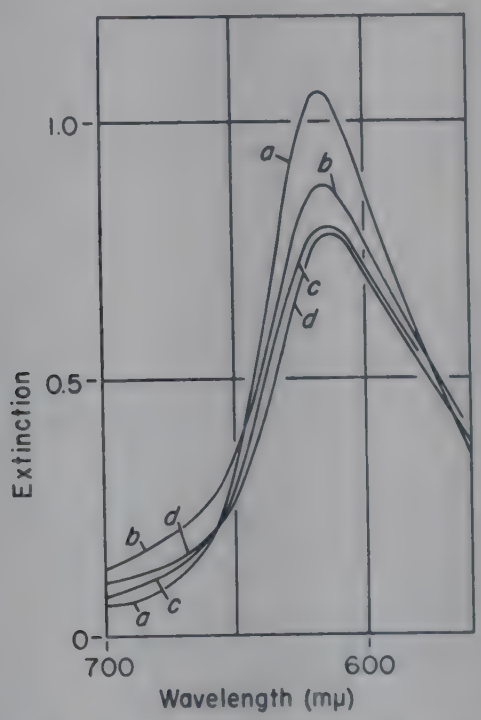


FIG. 5. The vitamin A_1 of the retinas of marine fishes. Spectra of the antimony chloride tests with extracts of bleached retinas reveal the absorption band maximal at 615–620 mμ characteristic of vitamin A_1. This result has been obtained with a wide variety of bony and elasmobranch fishes, and is characteristic also of land vertebrates. Two wrasse-fishes (Labridae), however, the cunner and tautog, though wholly marine, are exceptional in having a predominance of vitamin A_2 in their retinas. KEY: (*a*) sea bass; (*b*) scup; (*c*) sand flounder; (*d*) sea robin. (From Wald, *4, 5, 30.*)

another vitamin A_1 photopigment with λ_{max} about 520 mμ. Recently also, P. K. and P. S. Brown of our laboratory have examined the visual pigments of a number of European labrid fishes (39). *Crenilabrus festivas* and *C. massa* had porphyropsins, based upon retinene$_2$, with λ_{max} 513 mμ. *Labrus merula* yielded a visual pigment with over-all λ_{max} 498 mμ, which by differential bleaching was resolved into a rhodopsin with λ_{max} about 492 mμ and a porphyropsin (vitamin A_2) with λ_{max} near 510 mμ. It will be noted that the porphyropsins of these labrid fishes tend to lie at considerably lower wavelengths than the porphyropsins of freshwater fishes, perhaps because they contain opsins typical of marine fishes from moderate depths (see below).

In summary, marine fishes characteristically possess rhodopsins; the only present exceptions having been found among the Labridae and closely related Coridae, some of which possess predominantly porphyropsins. All the mammals and birds yet investigated use vitamin A_1 alone in vision, and ordinarily make from it rhodopsins with λ_{max} at 500 ± 2–3 mμ (chicken, pigeon, owl, cow, rabbit, pig). Curiously, man is exceptional with λ_{max} 493 mμ (*24*); and it is reported that Crescitelli has found the λ_{max} of opossum rhodopsin at the same wavelength (*40*). Recently Dowling in our laboratory has found rat rhodopsin to have λ_{max} 494 mμ (*41*).

The position of the reptiles requires further clarification. Some time ago we found the retinas of the freshwater turtles, *Pseudemys scripta* and *P. mobilensis*, to contain vitamin A_2, apparently with no admixture of vitamin A_1 (*12*). These retinas contain a great predominance of cones, and we were unable to obtain any measurable photosensitive pigment from them. We supposed from finding vitamin A_2 that the cone pigment is cyanopsin; and Granit's measurements (*13*) of the spectral sensitivity of cone vision in the Greek tortoise, *Testudo graeca*, showing a close correspondence with the absorption spectrum of cyanopsin, agree well with this supposition. In that case the few rods present in these retinas should be expected to contain porphyropsin.

On the other hand, alligator (*18*) and rattlesnake (*25*) retinas contain typical rhodopsins, based on vitamin A_1, and with λ_{max} about 500 mμ. Brown and Brown (*42*) have also found exclusively vitamin A_1, and as visual pigments rhodopsin (λ_{max} about 500 mμ) and iodopsin (λ_{max} about 560 mμ) in the retinas of the marine green turtle, *Chelonia mydas*.

Crescitelli (*25*) has found that in seven species of nocturnal geckos the visual pigments are rhodopsins, based upon vitamin A_1, yet with λ_{max} ranging between 518 and 528 mμ, overlapping therefore with λ_{max} characteristic of porphyropsins. Crescitelli suggests that this observation may support Gordon Walls's transmutation theory, according to which the geckos are derived from diurnal lizards which possessed cones; and their rods are to be regarded as secondarily transmuted cones. In that case their possession of a rhodopsin lying toward the red from the usual position might be a vestige from this ancestral condition. It may be added that a red-sensitive retinene$_1$ (cone?) pigment with λ_{max} 525–530 mμ has been found in the marine scup (*29*). It is possible that further exploration will reveal a more widely distributed class of rhodopsins in which the displacement of λ_{max} to wavelengths considerably longer than 500 mμ is evidence of "conishness."

The foregoing discussion can be summarized as follows: All known

freshwater fishes use vitamin A_2 in vision; all known marine fishes with the exception of a few Labridae and closely related Coridae use vitamin A_1. All known mammals and birds also use vitamin A_1 in vision, whereas the few reptiles examined divide in this regard: the alligator, rattlesnake, geckos, and marine turtles use A_1; some freshwater turtles, A_2. In every case the visual pigment has been found to be formed from whatever vitamin A the retina possesses; but owing to rods and cones possessing different opsins, and the opsins of different species sometimes introducing wide departures of λ_{max} from the usual positions, a degree of scatter and consequent confusion may be introduced by the visual pigments themselves that is avoided if one regards simply the retinal vitamins A. Yet the visual pigments also for the most part present us with a simple distribution. The few aberrations that have appeared among them have taken on exaggerated importance as workers grow bored with the prevalent forms, and concentrate their attention and energies on discovering exceptions.

IV. Euryhaline Fishes

The almost precise division between freshwater and marine fishes, the former using exclusively vitamin A_2 and the latter almost exclusively vitamin A_1 in vision, raised acutely the question of those fishes which are neither wholly freshwater nor marine, but migrate between both environments. Before considering the distribution of visual systems in these forms, it will be necessary to make plain their biological status (*30*).

All aquatic animals can be divided into forms restricted to narrow ranges of salinity, called *stenohaline*, of which there are two groups: freshwater and marine; and forms that range as adults through wide ranges of salinity, called *euryhaline*. The euryhaline fishes in turn are divided into two groups, called *anadromous* and *catadromous*, meaning literally upstream and downstream, referring to the direction of the spawning migration. Thus a typical anadromous fish is the salmon, which having grown up in the sea, migrates upstream to spawn; whereas the classic example of a catadromous fish is the "freshwater" eel, *Anguilla*, which having matured in fresh water migrates seaward to spawn.

Too much emphasis has been placed in the past upon the migrations of these animals. So far as known none of them needs to leave its spawning environment to complete a normal life cycle. The spawning environment itself is in every instance fixed; but migration away from it among adult fishes is nothing more than a privilege which some forms exploit frequently and others rarely. It is more meaningful to define an ana-

dromous fish as one that, having spawned in fresh water, has the capacity as an adult to migrate to the sea. A catadromous fish is one that, having spawned in the sea, is able as an adult to migrate into fresh water (*30*).

The examination of visual systems in such forms some years ago revealed the following situation. Members of three genera of salmonids (the Eastern brook trout, the Pacific coast rainbow trout, and the Pacific king salmon) all possess in their retinas mixtures of vitamins A_1 and A_2, of rhodopsin and porphyropsin, predominantly the latter—the form characteristic of the spawning environment. Conversely the freshwater eel has a mixture of both visual systems in which vitamin A_1 and rhodopsin predominate (Fig. 6) (*30, 43*).

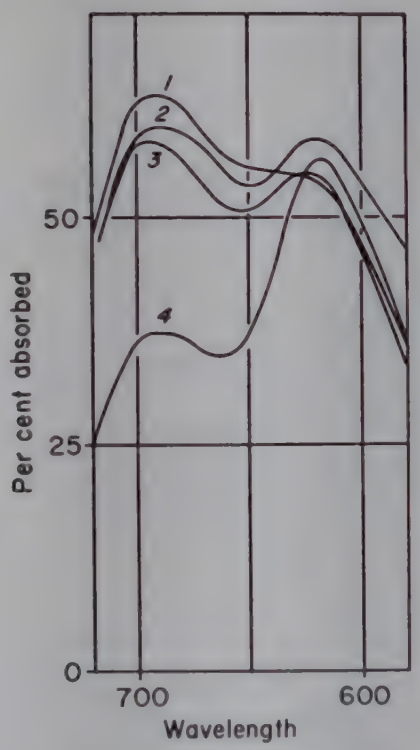


FIG. 6. Retinal vitamins A in euryhaline fishes. Spectra of antimony chloride tests with extracts of bleached retinas from (1) chinook salmon, (2) rainbow trout, (3) brook trout, and (4) American "freshwater" eel. All these tissues contain both vitamins A_1 and A_2, the anadromous salmonids a predominance of vitamin A_2, and the catadromous eel a higher proportion of vitamin A_1. (From Wald, *4, 5, 30.*)

My old observations on the vitamins A and visual pigments of the American freshwater eel require restatement on the basis of new information. I had found that the proportion of A_1 to A_2 in the retinas I examined was 62:38, stated in terms of the relative extinctions at 618 and 696 mμ in the antimony chloride test (*30*). It has since been determined that mole for mole, vitamin A_1 yields in this test an extinction 1.18 times as high as A_2, so that on a molar basis the ratio of vitamins A in the eel retina is reduced from the above figure to $A_1:A_2 = 58:42$. Paul Brown in

our laboratory has recently made an extensive survey of this ratio in
retinas of individual animals (*44*). He finds a wide range of molar ra
of A_1:A_2, from 25:75 to 65:35, with an average value (including
above observations) of A_1:A_2 = 45:55. However he has found also
porphyropsin possesses a molar extinction only about 3/4 that
rhodopsin. The ratio of the extinctions of rhodopsin:porphyropsin
these retinas is therefore on the average about 1.1:1. That is, ther
on the average a slight preponderance of vitamin A_2, and a slight
ponderance, extinction-wise, of rhodopsin, in the *Anguilla* retina.
Brown and Brown (*39*) have found the European *Anguilla* to resem
the American in this regard.

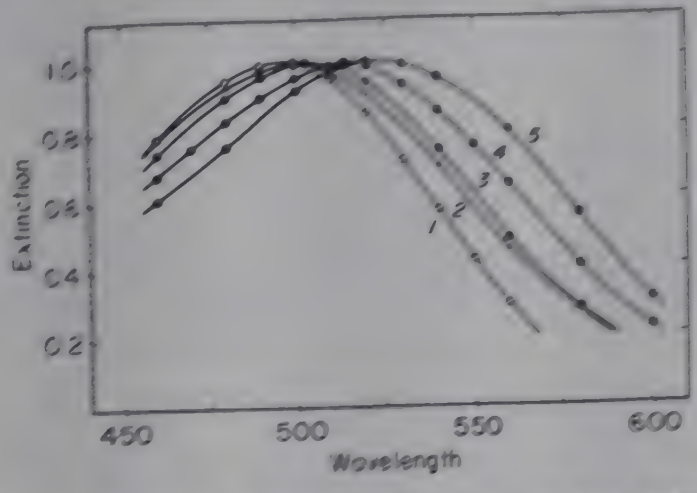
The anadromous alewife, *Pomolobus pseudoharengus*, and w
perch possess vitamin A_2 and porphyropsin alone (*30*, *43*). The
manently brackish water killifish, *Fundulus heteroclitus*, has a mixtu
both vitamins A_1 and A_2, and of rhodopsin and porphyropsin, predo
nantly the former, like the eel (*43*). All the euryhaline forms exam
followed the simple rule that all of them possess either predomina
or exclusively the vitamin A and visual system characteristic of
spawning environment.

These observations have been confirmed by a number of work
Kampa (*34*) on the European trout (λ_{max} 510 mμ); Collins and Mo
(*35*) on the char, an anadromous salmonid (λ_{max} 514 mμ); Bridges
on the rainbow trout. Carlisle and Denton (*46*) have found λ_{max} of
visual pigment in the immature European freshwater eel (*Ang
anguilla*) about where we found it in the American species, but I
introduced a further development with regard to this animal, of w
more below.

Munz (*47*) has reported measurements on a "euryhaline" fish,
mudsucker (*Gillichthys mirabilis*), which though possessing a ph
pigment with the intermediate λ_{max} 512 mμ, derives this wholly f
vitamin A_1. It is not clear why Munz speaks of this fish as euryha
He cites earlier work on this species by Kampa (*34*) and Weisel
both of whom speak of it as a marine teleost. Munz cites Weisel to
effect that the mudsucker can tolerate salt concentrations "both al
and below that of sea water." I know of no marine fish unable to do
same. The observation that the rhodopsin of this animal lies unusu
high in λ_{max} among marine fishes is interesting, but has little to do
the present problem.

In summary, the euryhaline fishes that have been examined fo
the rule already stated: they contain in their retinas either mixture
vitamins A_1 and A_2 in which the vitamin A ordinarily characteristic
the spawning environment tends to predominate, or that vitamin A al

known cases rhodopsin and porphyropsin are present in the same
rtions as the retinal vitamins A from which they are derived. In
quence of these relationships one can observe a regular duplicate
of λ_{max} in the total visual pigment of the retina, depending on the
rtions of rhodopsin and porphyropsin that are present, and cor-
d to a surprising degree with ecological habit. This is illustrated
g. 7. Beginning with the wholly marine spiny dogfish, which pos-



g. 7. Spectra of the visual pigments of various fishes, illustrating the transi-
rom an exclusively rhodopsin to an exclusively porphyropsin system, correlated
alinity relations. The permanently marine dogfish (1) possesses rhodopsin alone
approx. 497 mμ); the catadromous eel (2) and brackish-water killifish (3),
minantly rhodopsin; the anadromous brook trout (4), predominantly por-
psin, and the anadromous white perch (5), wholly porphyropsin (λ_{max} 522
(From Wald, 43.)

s typical rhodopsin (λ_{max} 498 mμ), one goes to the catadromous eel
brackish water killifish (λ_{max} 498–505 mμ), marine fishes which can
de fresh water, to the anadromous brook trout (λ_{max} 510–515 mμ),
shwater fish that can enter the sea; and finally to the white perch,
her anadromous fish that possesses exclusively porphyropsin (λ_{max}
mμ) (43).

s this pattern of visual systems determined genetically or by the
ronment? A first answer to this question is provided by those labrid
corid fishes which, though wholly marine, possess porphyropsin.
it is the euryhaline fishes, since they can be observed in both en-
nments, which demonstrate most clearly that to a first approximation
pattern of visual systems is independent of the immediate environ-
t. The salmonids which we found to have both systems mixed in
retinas had never been out of fresh water. Alewives just coming
fresh water from the sea on their spawning migration have vitamin
nd porphyropsin alone. Eels taken from a landlocked freshwater

pond have been found to contain in their retinas a predominance of vitamin A_1 and rhodopsin; and so on.

It is this realization that makes one look more to phylogeny than to physiology for a possible ordering and rationalization of the distribution of visual systems. Perhaps better said: there is an order here that goes with the ecology, but with the genetically determined rather than the casual ecology. The euryhaline fishes are intermediate not alone in their ecological relations and in their retinal patterns of vitamin A, but in both display a genetic primacy of the spawning environment. It is this issue of the spawning environment that raises a further problem.

V. Amphibia: Metamorphosis of Visual Systems

If spawning in fresh water is a dominant consideration in deciding the pattern of visual systems, then such an amphibian as the frog should possess predominantly porphyropsin. Instead it has rhodopsin; indeed rhodopsin was first discovered in the rods of frogs.

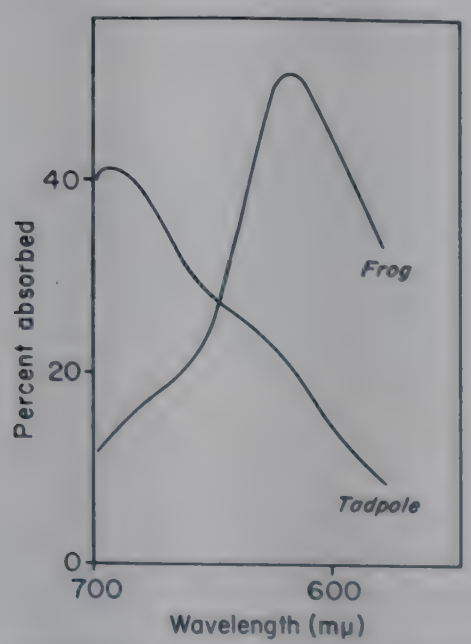


FIG. 8. Biochemical metamorphosis of visual systems in the bullfrog, *Rana catesbiana*. The tadpole just entering the metamorphic crisis has in its retina vitamin A_2 (i.e., porphyropsin) with only a trace of vitamin A_1 and rhodopsin; whereas the newly emerged froglet has just the reverse pattern. (From Wald, 5.)

This dilemma was resolved with the discovery that in the bullfrog, *Rana catesbiana*, tadpoles just about to enter the metamorphic climax have almost wholly porphyropsin accompanied by only a trace of rhodopsin, whereas newly emerged frogs have transferred almost wholly to rhodopsin, with just a trace of porphyropsin (Figs. 8, 9) (5, 49).

Wilt (50) has recently confirmed this observation, and has found that it can be brought about prematurely, as can the anatomical changes of metamorphosis, by treatment of the tadpoles with thyroxine. On the other hand, Collins *et al.* (51) found rhodopsins in both tadpoles and

adults of the European frogs, *Rana temporaria* and *R. esculenta;* here such a metamorphosis of visual systems apparently does not occur.*

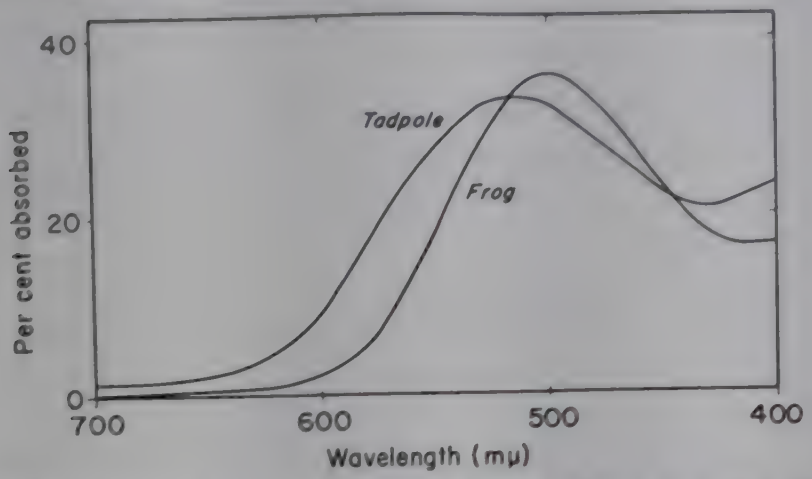


FIG. 9. Visual pigments extracted from tadpoles about to enter the metamorphic climax and newly emerged frogs (*Rana catesbiana*). The tadpole possesses predominantly porphyropsin (λ_{max} 516 mμ), the frog predominantly rhodopsin (λ_{max} 500 mμ). (From Wald, 5.)

Comparable observations have been made on the urodele amphibian, *Diemyctilus viridescens*, the common New England spotted newt (*5, 49*) but these introduce a new consideration. This animal in its life cycle undergoes two striking metamorphoses: first from the larval tadpole to the wholly land-living red eft; then some one and a half years later, to the sexually mature, water-living adult. The red efts that I have examined possess in their retinas mixtures of vitamins A_1 and A_2, predominantly the former, whereas the sexually mature adults have either mixtures in which vitamin A_2 predominates, or the latter alone (Fig. 10). The transformation here is in the reverse direction from that found in the frog; but this is what I have called elsewhere a "second metamorphosis," and represents a characteristic reversal of the first metamorphosis, and to some degree a return toward the larval condition (*52, 53*).

The permanently larval urodele, *Necturus*, the mud puppy, has also been examined. The adult retina contains primarily vitamin A_2, like a larval frog (*5, 49*).

A particularly interesting case is offered by the frog *Xenopus*, the so-called clawed toad, an animal which though it metamorphoses never ordinarily leaves the water. Mature examples of this form have in their retinas mixtures of both visual systems, in which porphyropsin and vitamin A_2 greatly predominate (*54*).

It appears therefore that the amphibia, like the euryhaline fishes,

* *Note added in proof:* According to F. Crescitelli (*35a*) this is true also of the toad, *Bufo boreas*, which possesses rhodopsin (λ_{max} 502 mμ) in both tadpoles and adults. On the other hand Crescitelli finds that the Pacific tree frog (*Hyla regilla*) metamorphoses from porphyropsin in the tadpole (λ_{max} 524 mμ) to rhodopsin in the adult (λ_{max} 503 mμ).

use both vitamins A_1 and A_2 in vision, and to this degree he inter-
mediate between freshwater fishes and land vertebrates as the euryhaline
fishes do between freshwater and marine fishes. The anatomical meta-
morphoses which are so striking in the amphibia are accompanied on
occasion by biochemical metamorphoses of their visual systems. Such
changes can be found not only at the first metamorphosis from the larva

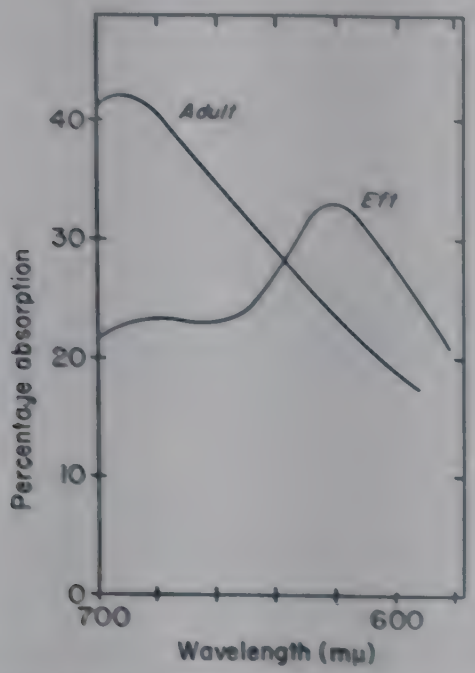


FIG. 10. Second metamorphosis of visual systems in the New England spotted
newt, *Diemictylus viridescens*. Retinas of the land-living red eft contain predomi-
nantly vitamin A_1 with a minor admixture of A_2; retinas of water-phase, sexually
mature adults contain predominantly or exclusively vitamin A_2. (From Wald, 53.)

to the adult stage, but, opposite in direction, at the second metamor-
phosis which brings the sexually mature animal back to the spawning
environment, so closing the life cycle (53). Even some of the aberra-
tions of amphibian development are paralleled closely by the pattern of
visual chemistry: the permanently larval mud puppy retaining the
characteristically larval vitamin A_2, and the permanently aquatic
Xenopus the same.

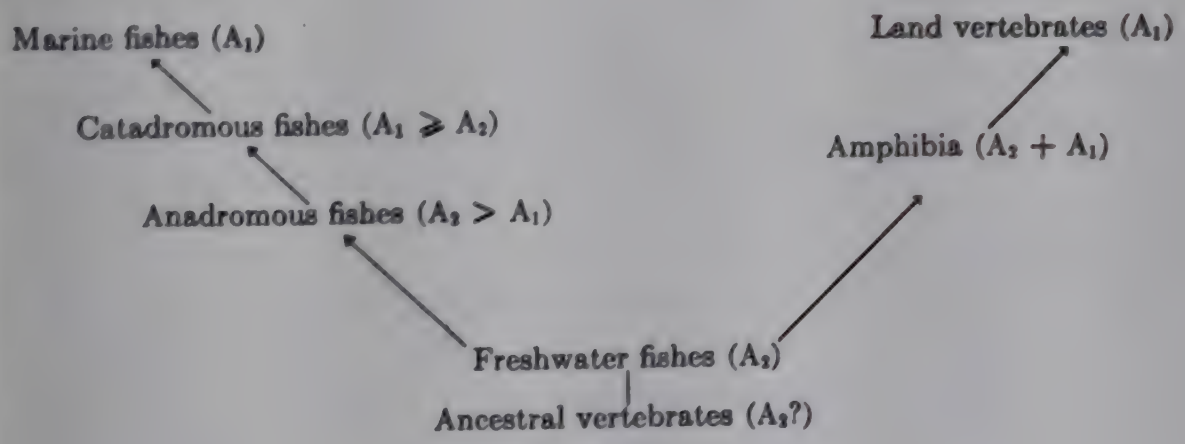
VI. Vertebrate Visual Systems: Habitat Relations and Phylogeny

What emerges from this discussion is the conclusion that there exists
an astonishingly consistent relationship between the habitat and the use
of vitamins A_1 and A_2 in vision. This brings together the marine fishes
and land vertebrates in their almost exclusive use of vitamin A_1 in
vision, reserves to freshwater fishes a perhaps exclusive use of vitamin
A_2, and finds both vitamins in regular use by the forms which are transi-
tional in habitat — the euryhaline fishes and the amphibia.

Since all these patterns are genetic, one can hardly avoid asking whether this distribution has some connection with phylogeny. This is always a dangerous question, but worth asking if only for the stimulation it affords.

Many paleontologists agree that the vertebrate stock arose in fresh water. The whole of vertebrate evolution, from this point of view, may be regarded as involving occasional migrations from this ancestral environment in two directions: to the sea, to produce the marine fishes; and to land, to yield the reptiles, birds, and mammals. Interpolated between freshwater and marine fishes are the euryhaline forms. Interpolated between freshwater fishes and land vertebrates are the amphibia.

Let us for the moment take the freshwater fishes to represent the ancestral form of visual system. In that case the distribution of visual systems according to habitat becomes a phylogenetic tree of the form shown in Diagram III.



(III)

The particular violence to phylogeny introduced in this scheme involves the position of the freshwater fishes. Paleontologists are inclined to regard most modern freshwater bony fishes, not as ancestral forms, but as late derivatives from marine species which have secondarily invaded fresh water. That is, they believe that vertebrates, having originated in fresh water and from there having colonized the sea, have at times returned to fresh water, and that the usual types of freshwater bony fishes have this origin. How then can we take the properties of modern freshwater fishes to represent an ancestral condition? Only on the somewhat far-fetched assumption that whenever marine fishes return to fresh water they revert to the ancestral freshwater type of visual system. What genetic sense can be made of such a hypothesis remains to be determined.

I should like to re-emphasize that the correlation of the retinal vitamin A pattern with the habitat relations of contemporary animals is

clear; that these relations are determined genetically is also clear. What is and will remain open to doubt is the arrangement of this information in the form of a phylogenetic tree. All phylogenetic trees are far-fetched; by reiteration they come to seem less far-fetched. The most they can hope to do is to provide an orderly arrangement of observations, and perhaps to stimulate new observations. I do not suppose that the above arrangement represents more than this. It is not universally agreed that the vertebrates arose in fresh water, and the weakness of equating ancestral vertebrates with present freshwater teleosts has already been noted. One is on surer ground in believing that freshwater fishes gave rise to amphibia, and those in turn to reptiles and other land vertebrates.

Could one arrange the same data otherwise and achieve equal order? Suppose, for example, one assumes that the ancestral vertebrates used vitamin A_1 in vision. Then one must somehow get to freshwater fishes with A_2, from these to amphibia with A_2 and A_1, then back to A_1 in the land vertebrates. This has the unattractive feature of vacillating where the former scheme presents a continuous development, yet otherwise represents the observed biochemical and ecological relationships equally well.

VII. Lampreys

An opportunity to come further with these problems is offered by the cyclostomes. These members of the ancient subclass Agnatha, the jawless vertebrates, include the most primitive living vertebrates. Among them the hagfishes are blind, and so cannot help us. The lampreys, however, have a reasonably well-formed eye.

Some years ago the author examined the retinas of the sea lamprey, *Petromyzon marinus*, an anadromous form that has a life cycle like the salmon. I found them to contain both vitamins A_1 and A_2, with a great predominance (about 90%) of A_2 (55). I concluded from this and from earlier reports of the color of lamprey retinas that this animal possessed primarily porphyropsin. It seemed to me that this observation lent support to the view that porphyropsin is the primitive vertebrate visual pigment. Clearly the first scheme of vertebrate phylogeny presented above would accept this datum smoothly, whereas it would be an added embarrassment for the second.

Recently, however, Crescitelli (56) extracted the visual pigment from retinas of this species of lamprey, and found the pigment to be rhodopsin. Crescitelli noted that this observation favored the view that rhodopsin rather than porphyropsin is the primitive type of vertebrate pigment.

Crescitelli's animals, however, were newly metamorphosed from the larval condition, on their journey downstream, whereas mine had been at the other end of their life cycle, mature animals migrating upstream from the ocean to spawn. In the following year, on collecting animals in both these stages, I found that both Crescitelli and I had been correct. The downstream migrants possess vitamin A_1 and rhodopsin alone; the upstream migrants almost entirely vitamin A_2 and porphyropsin (Figs. 11, 12). The lamprey exhibits a biochemical metamorphosis of

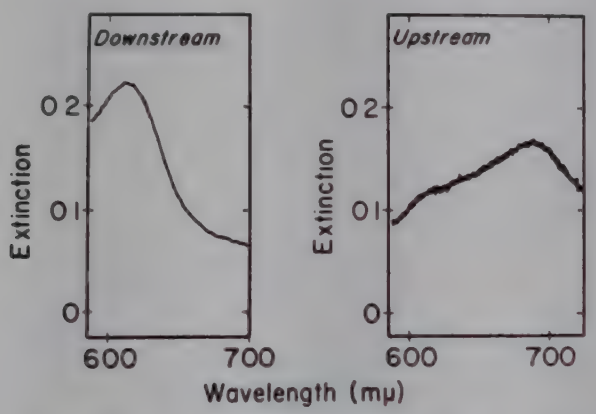


FIG. 11. Antimony chloride tests with extracts of the bleached visual pigments from downstream migrant sea lampreys and upstream migrant sea lampreys. The test for the downstream migrants displays only the absorption band maximal at 618 mμ characteristic of vitamin A_1. The test for the upstream migrants shows the presence of vitamin A_2 (λ_{max} 690 mμ), accompanied by a trace of vitamin A_1 (λ_{max} 615 mμ). (From Wald, 52.)

visual systems related to its life cycle as are similar metamorphoses in the amphibia (52).

It should be noted at once that this again is a *second metamorphosis,* marking the return to the spawning environment, like that already encountered in the newt. In both cases the transition is from vitamin A_1 to A_2, but in a second metamorphosis one expects such transitions to represent just the reverse of recapitulations (53). That is, in such cases one expects the sexually mature animals to have reverted toward the larval condition, creating the presumption that the larval visual system in this lamprey should be based upon vitamin A_2. Since the larval lamprey is blind, this cannot be tested. It should be possible however to test the general validity of this argument in other animals that go through two metamorphoses and possess eyes as larvae: eels, for example, and newts.

Other lampreys have not yet been examined in this detail. Kühne (57) reported finding the retinas of *Lampetra fluviatilis,* another anadromous form, feebly purple in color, inclining toward violet or bluish, suggesting that this animal contained porphyropsin. Crescitelli

(*56*) reports measurements on one extract of five sexually mature Pacific coast lampreys, *Entosphenus tridentatus*, which appeared to contain rhodopsin. In this, *Entosphenus* may resemble the bullfrog, which goes over to rhodopsin at the first metamorphosis and retains it thereafter, whereas *P. marinus* like the newt changes visual systems in its second metamorphosis.

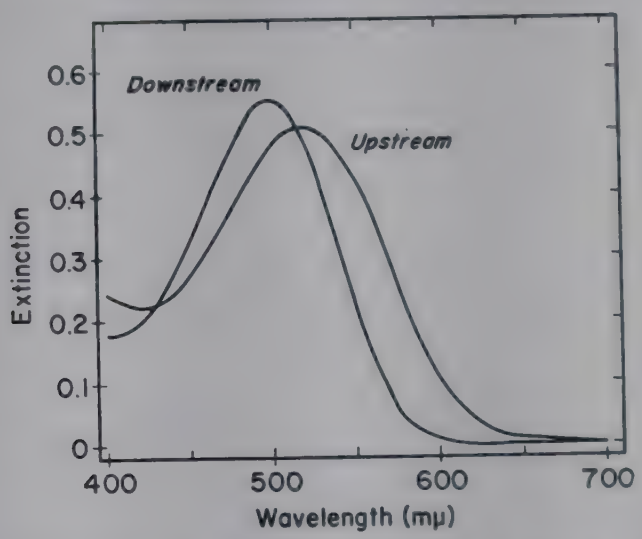


FIG. 12. Second metamorphosis of visual systems in the sea lamprey, *Petromyzon marinus*. The downstream migrants, having recently undergone the first metamorphosis from the larval stage, possess rhodopsin. The sexually mature adults, migrating upstream from the sea to spawn, have almost only porphyropsin. (From Wald, 52.)

More important, however, than any of these details is the realization that this most primitive group of vertebrates does possess vitamin A_2 and porphyropsin; and this I believe fits better with the view that porphyropsin is the primitive vertebrate visual pigment, and with the phylogenetic order presented in Diagram III above.

VIII. Deep-Sea Fishes

Denton and Warren (*26*) have recently made the extraordinary discovery that whereas surface forms of marine fish have rhodopsins with λ_{max} near 500 mμ, those found at depths of about 500 meters (about 275 fathoms) have instead pigments which they describe as golden in color, and for which they suggest the name chrysopsins, i.e., "visual gold," with λ_{max} near 480 mμ. These workers did not extract the pigment, but measured its difference spectrum in fresh retinas.

Munz (*40*, *58*) has confirmed this observation, extracting the visual pigments from fish taken at night at 280–380 fathoms, the λ_{max} of which in solution ranged between 478 and 490 mμ. Munz found one species in which a pigment with λ_{max} 478 mμ occurs together with ordinary

rhodopsin of λ_{max} 500 mμ. All these pigments yielded retinene$_1$ on bleaching.

Wald and co-workers (59) have made two further contributions to this development. They have shown that fishes which frequent depths between the surface and about 200 fathoms tend to possess visual pigments intermediate in λ_{max}. Even with the present small sample one perceives some degree of regularity of transition of the absorption spectra of the visual pigments of marine fishes, correlated with their habitual ranges of depth. A group of such absorption spectra is shown in Fig. 13, and the relation between the position of λ_{max} and depth is

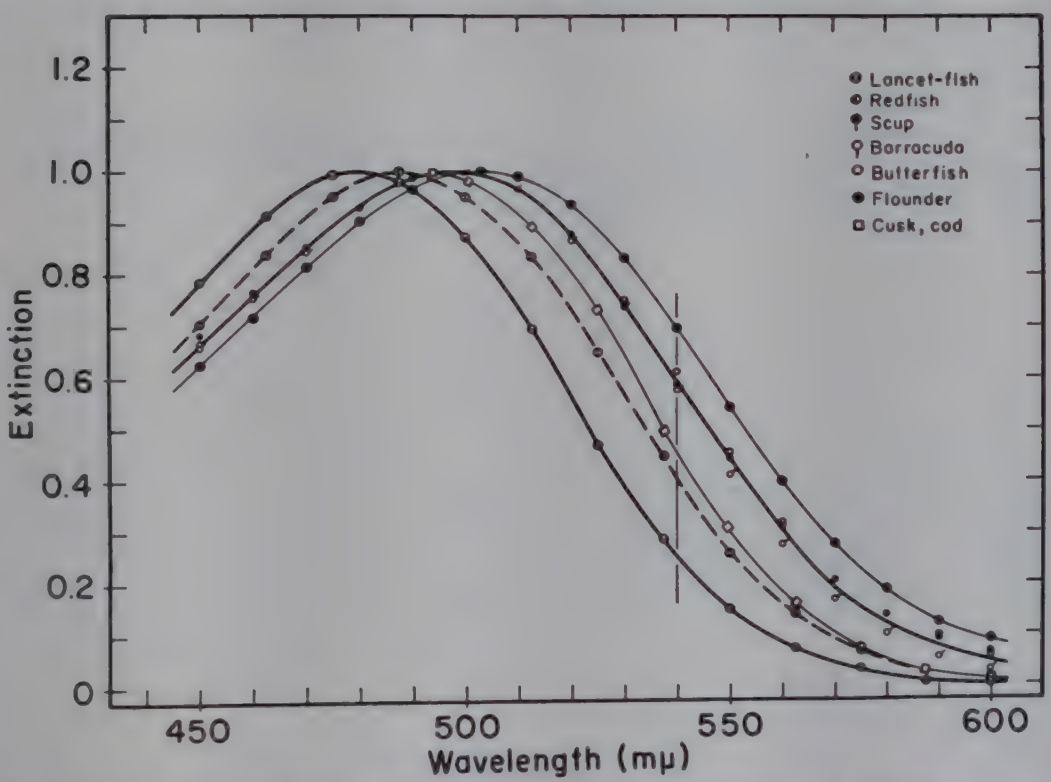


FIG. 13. The rhodopsins of fishes taken at various depths in the sea. That of the lancetfish, found ordinarily below 200 fathoms, has λ_{max} about 480 mμ; those of surface forms (scup, butterfish, barracuda, flounder) have λ_{max} 498–503 mμ. The cusk and cod (mean summer depths 40–50 fathoms) have λ_{max} 494–496 mμ; and the redfish (mean depth about 100 fathoms) has λ_{max} 488 mμ. The vertical line at 540 mμ indicates a more accurate index of this shift of spectrum, the ratio of extinctions at 540 mμ and at the maximum (E_{540}/E_{max}). (From Wald *et al.*, 59.)

shown in Fig. 14. Fishes roam widely in depth, and it is already clear that a wide spread of λ_{max} can be found at all depths, so that the curves drawn in Fig. 14 claim to do no more than suggest the course of an emerging relationship; yet I think it is already probable that statistically such a relationship exists. The second contribution of these workers was the demonstration that the deep-sea pigments, like those of surface forms, have as prosthetic group neo-b retinene. The deep-sea lancet fish, for example, has a typical deep sea pigment with λ_{max} 480 mμ.

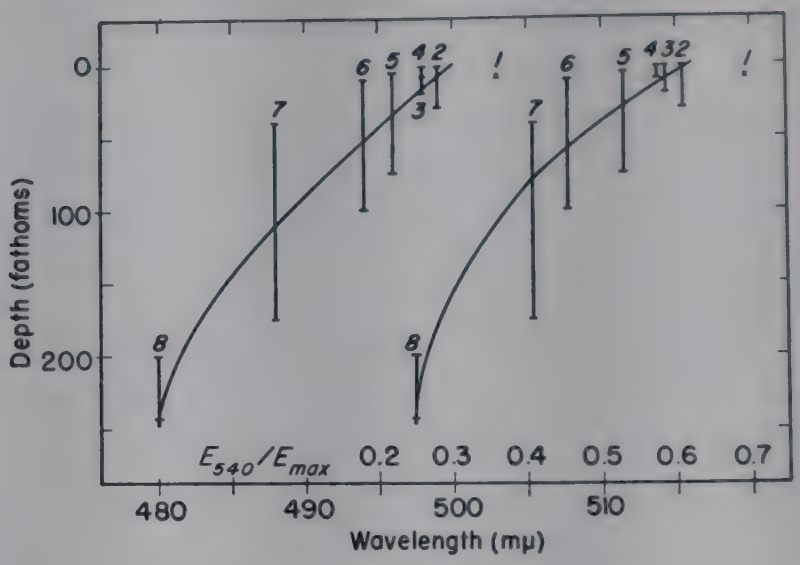


FIG. 14. Variation of absorption spectra of rhodopsins of marine fishes with summer range of depth. Left: λ_{max}; right: E_{540}/E_{max} (cf. Fig. 13). (From Wald *et al.*, 59.) The tabulation below provides a key to the figure.

Species	Summer range of depth (fathoms)	λ_{max} (mμ)	E_{540}/E_{max}
1. Summer flounder (*Paralichthys dentatus* Linnaeus)	2–10	503	0.695
2. Butterfish (*Poronotus triacanthus* Peck)	1–30	499	0.610
3. Scup (*Stenotomus versicolor* Mitchill)	1–20	498	0.586
4. Barracuda (*Sphyraena borealis* DeKay)	1–10[a]	498	0.575
5. Cod (*Gadus callarias* Linnaeus)	5–75	496	0.530
6. Cusk (*Brosme brosme* Müller)	10–100	494	0.455
7. Rosefish (*Sebastes marinus* Linnaeus)	40–175	488	0.407
8. Lancet-fish (*Alepisarus ferox* Lowe)	>200	480	0.250

[a] H. B. Bigelow, personal communication.

When it is bleached and neo-b retinene added in the dark, it regenerates with the same λ_{max}. All these pigments are identical with other rhodopsins in their prosthetic group, and owe their differences in spectrum entirely to the possession of different opsins. For this reason we prefer to call them *deep-sea rhodopsins*.

From this point of view, disregarding for the moment a few exceptional cases, one can recognize a major transition of rod visual pigments

in fishes from λ_{max} 522 to 500 mμ, which is correlated with salinity and depends upon the change of prosthetic group from retinene$_2$ to retinene$_1$ (Fig. 7), and a second major transition from λ_{max} 500 to 480 mμ, which is correlated with depth and depends upon the change of opsin (Fig. 13).

It seems probable that the rhodopsins of marine fishes do not greatly transcend the limits already disclosed (*Argyropxelecus affinis*, λ_{max} 478 mμ (*58*); *Gillichthys mirabilis*, a surface form, λ_{max} 512 mμ (*47*). As Denton and Warren indicate, light from the surface does not penetrate more deeply than the depths already sampled. These authors note also that the displacement of λ_{max} toward 480 mμ may have adaptive significance, since it is blue light of about this wavelength that penetrates most deeply into clear ocean waters. Indeed at still greater depths than those at which vision is possible with light penetrating from the surface, fishes must see mainly by bioluminescence; recent studies have shown that bioluminescent radiations also tend to be of maximal intensity at about 475–480 mμ (*60*).

Denton and Warren (*26*), having made their measurements directly on fresh retinas, have discovered also that deep-sea fishes tend to exhibit extraordinary densities of visual pigmentation. The change of extinction at about 485 mμ on bleaching they found in a few instances to be as large as 0.98–1.20, corresponding to absorptions of 90–94% of light of this wavelength reaching the retina. Through this device, more than through the shift of λ_{max}, some of the fishes that frequent great depths appear to achieve high visual sensitivity.

IX. Eels

We have already described finding in the American freshwater eel, *Anguilla rostrata*, mixtures of vitamin A_1 and A_2, and corresponding mixtures of rhodopsin and porphyropsin, so that the net λ_{max} of the visual pigment lies at 502–510 mμ. Recently Carlisle and Denton (*46*), working with the European *Anguilla anguilla*, have confirmed this result with so-called yellow eels that have not yet reached sexual maturity, in the same stage therefore as those used in our experiments. They find however that so-called silver eels, which are sexually mature and about to migrate oceanward to spawn, have changed to deep-sea rhodopsin, with λ_{max} about 485 mμ. This is therefore another and particularly interesting example of a *second* metamorphosis of visual pigments, involving in this case not a change of their prosthetic group, but a change of opsin.

My associates Paul and Patricia Brown (*61*) have recently reexamined this situation at the Stazione Zoologica in Naples. They found

yellow eels to possess various proportions of vitamins A_1 and A_2 in their retinas, and mixtures of rhodopsin and porphyropsin with net λ_{max} 504–513 mμ. The porphyropsin alone could be shown by differential bleaching in the presence of hydroxylamine to have λ_{max} 523 mμ; the rhodopsin, by regeneration with neo-b retinene was shown to have λ_{max} 501–502 mμ. As in the American species, in the average eel rhodopsin had a higher extinction than porphyropsin, but was present in a slightly lower *molar* concentration; a little more vitamin A_2 was present in the retina than A_1.

The silver eels however had entirely lost their vitamin A_2, and possessed vitamin A_1 alone in the retinas. This change seems to antedate the transition from ordinary to deep-sea opsin, for in various silver eels that had already lost their vitamin A_2 the λ_{max} of the visual pigment ranged between 500 mμ and 487 mμ, as though the opsin were in various stages of transition.

X. Invertebrates

Many of the lower invertebrates possess phototropic and phototactic reactions, and in a number of cases the action spectra associated with these have been determined. I know of no instance as yet, however, in which a reliable study of the spectral sensitivity has been coupled with the examination of the pigments involved. It is high time that such studies were undertaken and pursued systematically, for this is a field that offers abundant material and every promise of revealing new relationships.

It is only in the mollusks and arthropods that well-developed image-forming eyes are encountered. The microstructure of the photoreceptor organelles has lately been examined in representatives of both phyla, and found to present the same basic pattern in all. Whereas in vertebrate eyes the rod and cone outer segments are built of transverse layers (*62*), the fundamental structure of the rhabdome, the comparable organ in invertebrate eyes, consists of transverse parallel *tubules*, so that in longitudinal section, depending on the orientation, one sees either an appearance of layers because one is cutting along the tubules, or a honeycomb structure because one has cut across their ends. This arrangement has been shown in the rhabdomeres of the compound eyes of insects (*63*), in the arachnid *Limulus*, and a variety of crustacea (*64*), and in the so-called rods of the squid (*65*).

The visual systems of few arthropods and mollusks have yet been analyzed chemically, but all of them so far examined are based upon vitamin A_1 and retinene$_1$ alone. It should be noted at once that this basic similarity in composition to the systems of vertebrate vision does

not involve any genetic connection. The eyes of arthropods, mollusks, and vertebrates are entirely separate developments, each the product of its own evolution. No anatomical or embryological homologies connect these organs with one another; nor does anyone now suppose that either arthropods or mollusks were ancestors of the vertebrates. That all three phyla, with their very different eyes, should have come to the same substance, vitamin A—indeed, neo-b vitamin A—as the foundation of their visual systems poses in its most acute form the question: what constitutes the peculiar *fitness* of this molecule for vision? I have attempted to approach this question in the introductory section of the present paper.

The first such system to be worked out was that of the squid, *Loligo pealii*. Wald had found vitamin A_1 and retinene$_1$ in this retina, and showed that on illumination a small amount of retinene was liberated from a photosensitive combination with protein. Vitamin A itself did not appear to take part immediately in this transformation. The entire system seemed to involve only the reversible sequence (*66*):

$$\text{Photopigment} \overset{\text{light}}{\rightleftharpoons} \text{Retinene} + \text{Protein}$$

Bliss (*67*) later extracted a retinene-protein from the retina, which though stable in the light, decomposed in the dark. He believed this to be the visual pigment, and named it cephalopsin, but later analysis showed that it was in fact the metarhodopsin of the squid, and revealed a photosensitive rhodopsin as its precursor (*68*). A recent careful analysis by Hubbard and St. George (*69*) has now greatly extended our view of this system. Squid rhodopsin (λ_{max} 493 mμ) on irradiation yields at neutrality a mixture of the alkaline and acid forms of metarhodopsin (λ_{max} respectively 380 and 500 mμ). The pK of metarhodopsin is 7.7, so that at pH 6.5 or below practically all of this substance is in its acid form, with λ_{max} 500 mμ. It seems now that under physiological conditions the system goes no further, and that it may consist wholly of the reaction pair:

$$\text{Squid rhodopsin} \overset{\text{light}}{\rightleftharpoons} \text{Metarhodopsin}$$

for it is only at temperatures above 23°, and probably on denaturation of opsin, that metarhodopsin decomposes to retinene and opsin.

Kampa (*28*) reported the extraction from the eyes of the euphausiid shrimp, *Euphausia pacifica*, of a photosensitive pigment with λ_{max} of its difference spectrum at 462 ± 1 mμ. In these extracts she identified vitamin A, but did not detect retinene. Readers have drawn the inference that this pigment is made from vitamin A directly, rather than from

retinene, but this is not explicitly suggested by Kampa, and is intrinsically unlikely. It seems to me in view of what is said just below that the vitamin A found by Kampa in her extracts was also present before bleaching, and that her methods may not have been sufficiently sensitive to detect the retinene associated with the visual pigment.

It is a remarkable property of the invertebrates so far investigated that almost all their vitamin A is concentrated in their eyes. In the squid Wald was unable to detect a trace of vitamin A in whole bodies less the eyes (*66*). More than 90% of the total vitamin A in euphausiid crustacea is found in their eyes. Frequently no vitamin A at all can be found in other tissues, or in whole bodies less the eyes. The same is true of a number of decapod crustacea: lobster, green crabs, and shrimp (*70*).

It has recently been shown in the lobster (*71*) and the euphausiid *Meganyctiphanes* (*72*) that virtually all of the vitamin A stored in the eye is in the form of the hindered *cis* neo-b isomer. How these animals can form and maintain exclusively this relatively unstable configuration of vitamin A is an interesting problem, to which as yet we have no answer. The concentrations of vitamin A in these eyes are enormous. It seems probable to me that some of this substance that had been extracted from the eye along with the photosensitive pigment was the source of the vitamin A identified by Kampa (*28*) in her euphausiid extracts after bleaching.

Recently a photosensitive pigment has been extracted from the eye of the American lobster (*73*). It is interesting that in this instance, encouraged by the observation that rhabdomeres have a microstructure comparable with that of vertebrate outer segments, we isolated a suspension of rhabdomere fragments by the same procedures of differential centrifugation used to isolate the outer segments of rods and cones. A rhodopsin was extracted with λ_{max} 515 mμ, which on irradiation yielded an intermediate pigment (metarhodopsin) with λ_{max} 490 mμ, possessing a higher extinction than rhodopsin itself, and this decomposed slowly in the dark at pH 9.3 and 29° to retinene$_1$ and opsin. The process even at this high pH and temperature took hours to complete (half-time, 43 minutes); and probably involved the denaturation of opsin, as in the squid. That is, here again the system physiologically may consist mainly of the reaction:

$$\text{Lobster rhodopsin} \overset{\text{light}}{\rightleftharpoons} \text{Metarhodopsin}$$

Goldsmith (*74*) has succeeded in finding retinene$_1$ for the first time in an insect eye, that of the bee. By pursuing the protein that yielded retinene$_1$ on denaturation, he has extracted the first photosensitive pig-

ment from an insect eye. It has λ_{max} about 440 mμ, close therefore in the spectrum to the human violet receptor (*75*).

Brown and Brown (*27*) have examined the visual pigments of the European cuttlefish (*Sepia officinalis*) and octopus (*Octopus vulgaris*). Sepia rhodopsin has λ_{max} 492 mμ, and on irradiation yields metarhodopsin (acid form, λ_{max} 497 mμ; alkaline λ_{max} 380 mμ). Octopus rhodopsin has λ_{max} 475 mμ, like that of a deep-sea fish. On irradiation this goes to metarhodopsin (acid form, λ_{max} 502 mμ; alkaline λ_{max} 380 mμ).

To summarize these results: all these animals contain visual pigments based upon vitamin A_1 and retinene$_1$, comparable in their fundamental chemistry to vertebrate rhodopsins, and hence referred to here as rhodopsins. Ultimately these pigments must be derived from vitamin A_1 itself, and the organisms must possess mechanisms in the eye or elsewhere for oxidizing this to retinene$_1$, and hence also for reducing the latter. Yet no active alcohol dehydrogenase system has yet been found in an invertebrate eye. Nor has any one of these invertebrate systems—except apparently that of the bee—been found under physiological conditions to bleach so as to yield free retinene$_1$. On a short-term basis, they appear normally to operate with the simple reversible system:*

$$\text{Rhodopsin} \underset{}{\overset{\text{Light}}{\rightleftharpoons}} \text{Metarhodopsin}$$

XI. Summary and Conclusions

Virtually all these observations can be brought together in a diagram such as shown in Fig. 15.

It must be emphasized again that as a statement of the distribution of vitamins A among the visual systems of contemporary animals, and its relations to taxonomic and ecological groupings, this diagram correctly represents the observations. Its suggestion of phylogenetic sequence raises more stubborn questions, and will continue to do so.

Nevertheless, even in this sense such a diagram has much to tell us. It is sometimes argued that natural selection, based as it is upon chance variations, could not possibly have led to such intricate and effective structures as organisms possess; and a favorite structure cited in support of this view is the eye. "Think of the eye!" one hears it said, "Can you imagine chance variations ever giving rise to an eye?" The question is meant to be rhetorical. The proposition is intended to be obviously absurd.

My reading of evolution goes quite differently. An eye is possible

* *Note added in proof:* R. Hubbard and G. Wald (*Nature*, 1960, in press) have extracted a rhodopsin from the eye of the horseshoe crab, *Limulus polyphemus*, with λ_{max} about 520 mμ, and which bleaches in the light to retinene$_1$ and opsin.

to living organisms; the proof is that we have one. It is also enormously advantageous. Being both possible and advantageous, *given enough time*, it is inevitable.

The justification for the last statement is in the realization that three times upon this planet, in complete independence of one another, groups of living organisms have evolved intricate, image-resolving eyes. These are the arthropod eye, the cephalopod eye, and the vertebrate eye. No phylogenetic relationships connect these structures, no anatomical or embryological homologies exist among them. Each is an entirely independent development (*5b*).

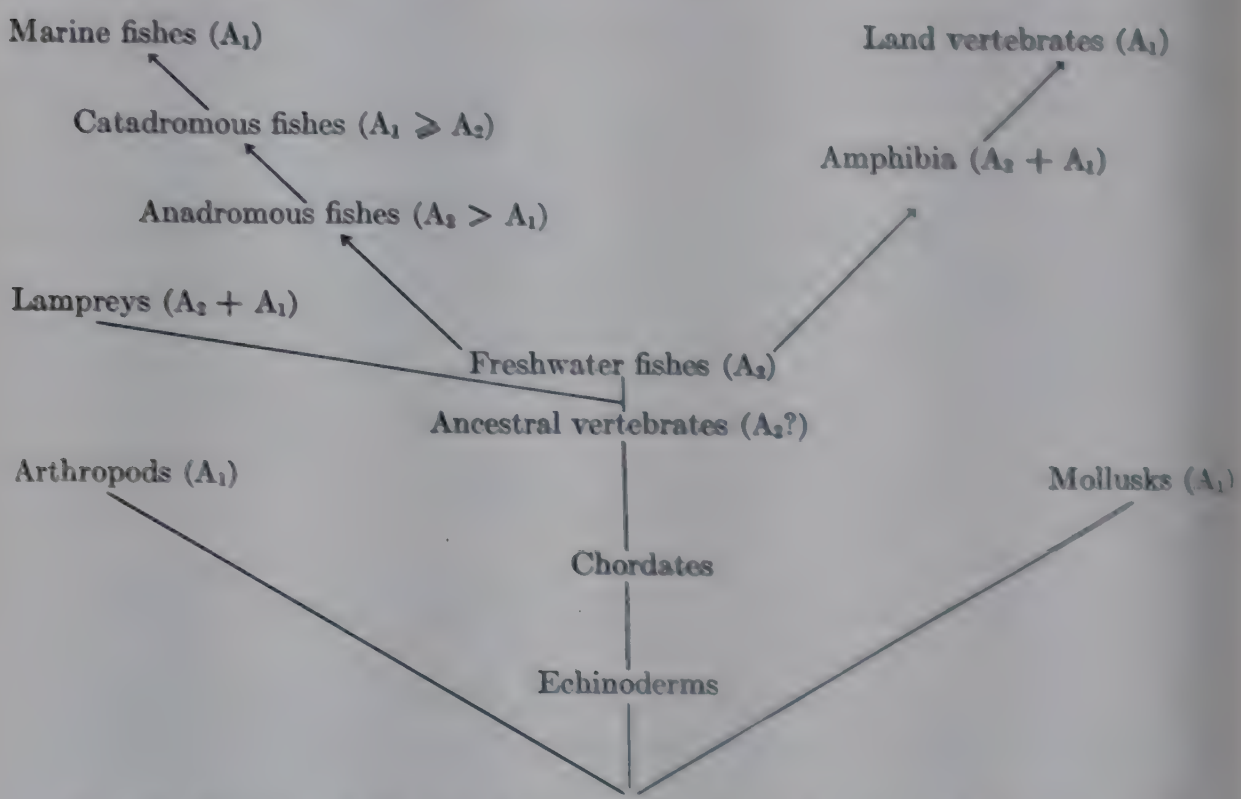


FIG. 15. Diagram of the distribution among animals of visual systems based on the vitamins A, arranged to suggest phylogenetic relationships. Well-formed eyes have appeared independently in three phyla: arthropods, mollusks, and vertebrates. All have come to use vitamin A_1 in vision, the vertebrates perhaps after beginning with vitamin A_2. (From Wald, 5.)

Anatomically these eyes differ greatly from one another. The cephalopod and vertebrate eyes have lenses, which throw inverted images of external objects on a retina. The lens is constructed very differently in the two phyla; the retina points toward the light in cephalopods and away from the light in vertebrates; microstructures of the light receptors are layers in vertebrates and bundles of tubules in cephalopods. The arthropod eye has an altogether different construction, depending not upon one lens but upon a battery of isolated optical systems, each complete in itself, to form an upright mosaic image.

Widely different though the anatomy and derivation of these eyes, all of them share very nearly the same chemistry. I see no alternative to believing that they achieved this chemistry independently of one another. I am willing to accept the implication that among the organic molecules that animals can make and that are made available to them by plants on this Earth, the carotenoids and vitamins A are particularly fitted for the construction of photoreceptor systems. I suggested at the beginning of this essay a number of aspects of the peculiar fitness of this family of substances for this function. I believe that for this reason animals, offered a wide choice of organic structures, have three times independently selected these, and have not to our knowledge ever selected any others.

Let me say this in a different way. Now that many of us believe that life is part of the order of nature, we see no escape from the conclusion that it is distributed widely in the universe, on perhaps many millions of planets like our own. I think that when biologists have had the opportunity to examine the fauna of other well-lighted planets, planets that have possessed life as has ours for perhaps several billion years, they will find eyes. In the eyes will be light-sensitive pigments, and these pigments will frequently, perhaps usually, have as prosthetic groups such polyenes as the carotenoids and vitamins A.

References

1. E. Pettit, *Astrophys. J.* **75,** 217 (1932).
2. E. S. Johnston, *Smithsonian Inst. Misc. Publs.* **92,** No. 11 (1934).
3. G. Wald, *Science* **101,** 653 (1945).
4. G. Wald, *Vitamins and Hormones* **1,** 195 (1943).
5. (*a*) G. Wald, *Harvey Lectures* **41,** 117 (1947); (*b*) G. Wald, *in* "Modern Trends in Physiology and Biochemistry" (E. S. G. Barron, ed.), p. 337. Academic Press, New York, 1952.
6. K. V. Thimann and G. M. Curry, this volume, Chapter 6.
7. G. Wald and H. Zussman, *J. Biol. Chem.* **122,** 449 (1938).
8. L. Zechmeister, *Chem. Revs.* **34,** 267 (1944).
9. R. Hubbard and G. Wald, *J. Gen. Physiol.* **36,** 269 (1952–1953).
10. R. Hubbard and A. Kropf, *Proc. Natl. Acad. Sci. U. S.* **44,** 130 (1958); A. Kropf and R. Hubbard, *Ann. N. Y. Acad. Sci.* **74,** 266 (1958).
11. G. Wald, *Am. J. Ophthalmol.* **40,** 18 (1955); G. Wald, *in* "Enzymes: Units of Biological Structure and Function" (O. H. Gaebler, ed.), p. 355. Academic Press, New York, 1956; R. A. Morton and G. A. J. Pitt. *Fortschr. Chem. org. Naturstoffe* **14,** 244 (1957).
12. G. Wald, P. K. Brown, and P. H. Smith, *Science* **118,** 505 (1953).
13. R. Granit, *Acta Physiol. Scand.* **1,** 386 (1941); R. Granit, *ibid.* **2,** 334 (1941).
14. G. Wald and R. Hubbard, *J. Gen. Physiol.* **32,** 367 (1948–1949); G. Wald. *Biochim. et Biophys. Acta* **4,** 215–228 (1950).
15. R. Hubbard and G. Wald, *Proc. Natl. Acad. Sci. U. S.* **37,** 69 (1951).

16. W. Oroshnik, P. K. Brown, R. Hubbard, and G. Wald, *Proc. Natl. Acad. Sc. U. S.* **42**, 578 (1956).
17. P. K. Brown and G. Wald, *J. Biol. Chem.* **222**, 865 (1956).
18. G. Wald, P. K. Brown, and D. Kennedy, *J. Gen. Physiol.* **40**, 703 (1936–1957).
19. R. Hubbard, *Nature* **181**, 1126 (1958).
20. E. E. Broda, C. F. Goodeve, R. J. Lythgoe, and E. Victor, *Nature* **144**, 7 (1939).
21. C. M. Radding and G. Wald, *J. Gen. Physiol.* **39**, 909 (1955–1956).
22. G. Wald, P. K. Brown, and P. H. Smith, *J. Gen. Physiol.* **38**, 623 (1954–1955).
23. G. Wald, P. K. Brown, and P. S. Brown, unpublished observations (1955–1958).
24. G. Wald and P. K. Brown, *Science* **127**, 222 (1958).
25. F. Crescitelli, *J. Gen. Physiol.* **40**, 217 (1956–1957).
26. E. J. Denton and F. J. Warren, *Nature* **178**, 1059 (1956); *J. Marine Biol. Assoc. United Kingdom* **36**, 651 (1957).
27. P. K. Brown and P. S. Brown, *Nature* **182**, 1288 (1958).
28. E. M. Kampa, *Nature* **175**, 996 (1955).
29. G. Wald, P. K. Brown, and P. H. Smith, *Federation Proc.* **13**, 316 (1954).
30. G. Wald, *J. Gen. Physiol.* **22**, 391 (1938–1939).
31. G. Wald, *J. Gen. Physiol.* **22**, 775 (1938–1939).
32. H. J. A. Dartnall, *J. Physiol.* **116**, 257 (1952).
33. H. J. A. Dartnall, *J. Physiol.* **128**, 131 (1955).
34. E. M. Kampa, *J. Physiol.* **119**, 400 (1953).
35. F. D. Collins and R. A. Morton, *Biochem. J.* **47**, 3 (1950).
35a. F. Crescitelli, *Ann. N.Y. Acad. Sci.* **74**, 230 (1958).
36. G. Wald, *Nature* **136**, 913 (1935); *J. Gen. Physiol.* **20**, 45 (1936–1937).
37. E. M. Kampa, *Yearbook Am. Phil. Soc.* p. 161 (1952).
38. F. W. Munz, *Nature* **181**, 1012 (1958).
39. P. K. Brown and P. S. Brown, unpublished observations (1958).
40. F. W. Munz, *J. Physiol.* **140**, 220 (1958).
41. J. E. Dowling, unpublished observations (1958).
42. P. K. Brown and P. S. Brown, unpublished observations (1958).
43. G. Wald, *J. Gen. Physiol.* **25**, 235 (1941–1942).
44. P. K. Brown, unpublished observations (1958).
45. C. D. B. Bridges, *J. Physiol.* **134**, 620 (1956).
46. D. B. Carlisle and E. J. Denton, *J. Physiol.* **139**, 8 (1957).
47. F. W. Munz, *J. Gen. Physiol.* **40**, 233 (1956–1957).
48. G. Weisel, Jr., *Physiol. Zool.* **21**, 40 (1948).
49. G. Wald, *Biol. Bull.* **91**, 289 (1946).
50. F. H. Wilt, *Develop. Biol.* **1**, 199 (1959).
51. F. D. Collins, R. M. Love, and R. A. Morton, *Biochem. J.* **53**, 632 (1953).
52. G. Wald, *J. Gen. Physiol.* **40**, 901 (1956–1957).
53. G. Wald, *Science* **128**, 1481 (1958).
54. H. J. A. Dartnall, *J. Physiol.* **125**, 23 (1954); G. Wald, *Nature* **175**, (1955); H. J. A. Dartnall, *J. Physiol.* **134**, 327 (1956).
55. G. Wald, *J. Gen. Physiol.* **25**, 331 (1941–1942).
56. F. Crescitelli, *J. Gen. Physiol.* **39**, 423 (1955–1956).
57. W. Kühne, *Untersuch. physiol. Inst. Univ. Heidelberg* **1**, 15 (1878).
58. F. W. Munz, *Science* **125**, 1142 (1957).
59. G. Wald, P. K. Brown, and P. S. Brown, *Nature* **180**, 969 (1957).
60. G. Clarke and G. K. Wertheim, *Deep-Sea Research* **3**, 189 (1956); G. Cl

and R. H. Backus, *ibid.* **4**, 1 (1956); E. M. Kampa and B. P. Boden, *ibid.* **4**, 73 (1957).
P. K. Brown and P. S. Brown, unpublished observations (1958).
F. S. Sjostrand, *J. Cellular Comp. Physiol.* **42**, 15 (1953).
T. H. Goldsmith and D. E. Philpott, *J. Biophys. Biochem. Cytol.* **3**, 429 (1957); J. J. Wolken, J. Capenos, and A. Turano, *ibid.* **3**, 441 (1957).
W. H. Miller, *J. Biophys. Biochem. Cytol.* **3**, 421 (1957); W. H. Miller, *Science* **126**, 1233 (1957).
G. Wald, R. C. C. St. George, and J. M. Goldstone, *Federation Proc.* **11**, 153 (1952); G. Wald, *Exptl. Cell Research (Suppl.)* **5**, 369 (1958).
G. Wald, *Am. J. Physiol.* **133**, 479 (1941).
A. F. Bliss, *J. Gen. Physiol.* **26**, 361 (1942–1943); A. F. Bliss, *J. Biol. Chem.* **176**, 563 (1948).
R. C. C. St. George and G. Wald, *Biol. Bull.* **97**, 248 (1949).
R. Hubbard and R. C. C. St. George, *J. Gen. Physiol.* **41**, 501 (1957–1958).
L. R. Fisher, S. K. Kon, and S. Y. Thompson, *J. Marine Biol. Assoc. United Kingdom* **31**, 229 (1952); **33**, 589 (1954); **34**, 81 (1955); L. R. Fisher, K. M. Henry, S. K. Kon, P. A. Plack, and S. Y. Thompson, *Biochem. J.* **61**, Proc. vi (1955).
G. Wald and S. P. Burg, *J. Gen. Physiol.* **40**, 609 (1956–1957).
G. Wald and P. K. Brown, *J. Gen. Physiol.* **40**, 627 (1956–1957).
G. Wald and R. Hubbard, *Nature* **180**, 278 (1957).
T. H. Goldsmith, *Proc. Natl. Acad. Sci. U. S.* **44**, 123 (1958); *Ann. N. Y. Acad. Sci.* **74**, 223 (1958).
W. S. Stiles, *J. Opt. Soc. Am.* **36**, 491 (1946); E. Auerbach and G. Wald, *Science* **120**, 401 (1954)

CHAPTER 8

erobic and Anaerobic Reactions of Inorganic Substances

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General Considerations

A. Introduction

Biochemical processes which produce free energy from inorganic bstances are found in a wide variety of living matter. The importance this source of energy, however, varies greatly from one end of the

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phylogenetic scale to the other. Only among bacteria are there organisms which can utilize the oxidation of inorganic substrates as their sole or even exclusive source of energy; according to the current nomenclature of nutritional types (Table I), they belong among the *chemolithotrophs*. These bacteria are often called *autotrophs*, a shorter term which signifies *self-supporting* and refers to the fact that, like phototrophic plants, they can grow on purely inorganic media in the absence

TABLE I
NOMENCLATURE OF NUTRITIONAL TYPES OF ORGANISMS BASED ON THEIR ENERGY SOURCES[a]

A. *Phototrophy:* Energy provided chiefly by photochemical reactions
   1. *Photo-lithotrophy:* Growth dependent on exogenous inorganic hydrogen donors (green and purple sulfur-bacteria)
   2. *Photo-organotrophy:* Growth dependent on exogenous organic hydrogen donors (non-sulfur purple bacteria)

B. *Chemotrophy:* Energy provided entirely by chemical reactions in darkness.
   1. *Chemo-lithotrophy:* Growth dependent on oxidation of exogenous inorganic substances (chemo-lithotrophic or "autotrophic" bacteria)
   2. *Chemo-organotrophy:* Growth dependent on oxidation or fermentation of exogenous organic substances ("heterotrophic" bacteria)

C. *Paratrophy:* Energy provided by host cell (intracellular parasitic bacteria and viruses)

[a] From "Heredity and Variation in Micro-organisms." *Cold Spring Harbor Symposia Quant. Biol.* **11**, 302 (1946).

of preformed organic matter, with CO_2 as the carbon source. This chapter will be concerned chiefly with the biochemical activities of these organisms, which are of considerable interest from the point of view of comparative biochemistry. The extensive literature on autotrophic bacteria has been reviewed by Stephenson (*1*), Umbreit (*2*), van Niel (*3*), Lees (*4*), and Foster (*5*), and by various contributors to an important symposium (*6*) held in 1954.

The oxidation of inorganic substances also plays an essential role in the metabolism of another group of bacteria, the *photo-lithotrophic* or photosynthetic bacteria. However, as will be seen later, the energy produced by these microorganisms from their inorganic substrates cannot be used by them for biosynthetic activities.

In the metabolism of heterotrophic bacteria, plants, and animals, there are several processes by which inorganic compounds—free or bound to organic groups—are oxidized. The best known reaction of this type is the oxidation by higher animals of the inorganic sulfur compounds produced at the terminal stages of organic sulfur metabolism. The amount of free energy produced in this way represents only a small

fraction of the total energy changes of heterotrophic organisms; it is not yet known whether this energy is available for biosynthetic functions.

Biochemical reactions yielding free energy from inorganic substrates are essentially oxidative and, by analogy with the nutritional nomenclature of bacteria, it is convenient to call them *litho-oxidations*. As in the case of biological oxidations of organic substances, they can be represented by the general equation

$$DH_2 + A \rightarrow D + AH_2 + \text{energy}$$

where DH_2 and D are the initial and final states of an hydrogen or electron donor, and A and AH_2 are the oxidized and reduced forms of an hydrogen or electron acceptor. Table II shows some examples of electron donors and acceptors involved in biological litho-oxidations.

TABLE II

ELECTRON DONORS AND ACCEPTORS USED BY SOME CHEMO-LITHOTROPHIC BACTERIA[a]

Organism	Energy source (electron donor)	Electron acceptor	End products of oxidation	
			From electron donor	From electron acceptor
Aerobic thiobacilli	S, $S_2O_3^{2-}$, H_2S, and other inorganic sulfur compounds	O_2	SO_4^{2-}	H_2O
Thiobacillus denitrificans	Same as above	NO_3^-	SO_4^{2-}	N_2
Thiobacillus ferrooxidans	$S_2O_3^{2-}$ or	O_2	SO_4^{2-}	H_2O
	Fe^{2+}	O_2	Fe^{3+}	H_2O
Nitrosomonas spp.	NH_4^+	O_2	NO_2^-	H_2O
Nitrobacter spp.	NO_2^-	O_2	NO_3^-	H_2O
Hydrogenomonas spp.	H_2	O_2	H_2O	H_2O
Desulfovibrio desulfuricans	H_2	SO_4^{2-}	H_2O	H_2S

[a] Modified from D. D. Woods and J. Lascelles, *4th Symposium Soc. Gen. Microbiol.* p. 1 (1954).

Concerning the nature of the electron acceptor, it should be pointed out that the classical distinction of aerobic and anaerobic processes is not as valid and useful in litho-oxidations as in the metabolism of organic compounds. Most of the enzymes catalyzing the oxidation of inorganic substrates are found in both aerobic and anaerobic organisms. An illustration is provided by hydrogenase, the enzyme which catalyzes the activation of molecular hydrogen:

$$H_2 \xrightarrow{\text{hydrogenase}} 2H^+ + 2e$$

In the strict aerobe *Hydrogenomonas*, this enzyme is coupled with a respiratory chain where oxygen is the terminal electron acceptor:

$$2H^+ + 2e + \tfrac{1}{2}O_2 \rightarrow H_2O$$

A hydrogenase is also present in the strict anaerobe *Desulfovibrio desulfuricans*, but in this case the terminal acceptor is sulfate which is reduced to sulfide:

$$2H^+ + 2e + \tfrac{1}{4}SO_4^{2-} \rightarrow \tfrac{1}{4}S^{2-} + H_2O$$

Moreover, *Thiobacillus denitrificans*, a bacterium which derives its energy from the litho-oxidation of thiosulfate to sulfate, can do so either aerobically in air or anaerobically if nitrate is present. These facts and many similar observations demonstrate that no sharp separation can be drawn between aerobic and anaerobic reactions.

B. Energetics of Biological Litho-oxidations

1. *Heat of Combustion and Free Energy Change*

The energetics of the biological litho-oxidations have been estimated by many authors from the heat of combustion of the substrate, i.e., from the total energy change of the reaction at constant temperature and pressure. According to the classical notation of Lewis and Randall (7) and Wurmser (8), the heat of reaction is expressed by the symbol $-\Delta H$.

A more precise and meaningful estimate is given by the free energy change ($-\Delta F^\circ$), i.e., the portion of the total energy exchanged which is convertible into work. At a given absolute temperature T, $-\Delta F^\circ$ and $-\Delta H$ are defined reciprocally by the equation

$$-\Delta F^\circ_T = -\Delta H_T - T\Delta S$$

where $T\Delta S$ is the energy not convertible into work and ΔS is the increase of entropy in the system.

Table III shows the numerical values of $-\Delta H$ and $-\Delta F^\circ$ for several litho-oxidations at the absolute temperature of 298°K (+25°C), under the standard conditions where the solids and liquids are dissolved in the aqueous phase at a concentration of 1.0 M and the gases are at a partial pressure of 1 atm. As pointed out by Marjorie Stephenson (1) in her book, the concentrations of the reactants have no effect on $-\Delta H$ but have a significant influence on the values of $-\Delta F$. When the reactants are highly diluted or at a very low partial pressure, as in most biological systems, $-\Delta F$ tends to increase and to approach the value of $-\Delta H$.

It may be seen from the data of Table III that many litho-oxidations are strongly exergonic reactions. However, none of them provides as

TABLE III

ENERGETICS OF BIOLOGICALLY CATALYZED LITHO-OXIDATIONS

Organism	Reaction	$-\Delta H_{298°}$ (kcal.)	$-\Delta F°_{298°}$ (kcal.)	References[a]
Nitrosomonas spp.	$NH_3 + \frac{3}{2}O_2 = HNO_2 + H_2O$	79		(*a*)
	$NH_4^+ + \frac{3}{2}O_2 = NO_2^- + H_2O + 2H^+$		66.5	(*b*)
Nitrobacter spp.	$HNO_2 + \frac{1}{2}O_2 = HNO_3$	21.6		(*a*)
	$NO_2^- + \frac{1}{2}O_2 = NO_3^-$		17.5	(*b*)
Thiobacillus thioparus	$5Na_2S_2O_3 + H_2O + 4O_2 = 5Na_2SO_4 + H_2SO_4 + 4S$	500.3		(*c*)
T. thiooxidans	$S + \frac{3}{2}O_2 + H_2O = H_2SO_4$	141.8	118	(*c*)
T. denitrificans	$5Na_2S_2O_3 + 8KNO_3 + 2NaHCO_3$			
	$= 6Na_2SO_4 + 4K_2SO_4 + 4N_2 + 2CO_2 + H_2O$	912.8	893	(*d*)
T. thiocyanooxidans	$NH_4CNS + 2O_2 + 2H_2O = (NH_4)_2SO_4 + CO_2$	220		(*e*)
T. ferrooxidans	$Fe^{2+} = Fe^{3+} + e$		11.3	(*f*)
Leptothrix ochracea	$4FeCO_3 + O_2 + 6H_2O = 4Fe(OH)_3 + 4CO_2$	81	40	(*b*)
Hydrogenomonas spp.	$H_2 + \frac{1}{2}O_2 = H_2O$	68.3	57.4	(*g*)
Desulfovibrio desulfuricans	$Na_2SO_4 + 4H_2 = Na_2S + 4H_2O$	60		(*h*)
	$SO_4^{2-} + 4H_2 = S + 4H_2O$		29.7	(*i*)
Beggiatoa spp.	$H_2S + \frac{1}{2}O_2 = H_2O + S$	32.5	41.5	(*b*)
	$S + \frac{3}{2}O_2 + H_2O = H_2SO_4$	141.8	118.5	(*b*)
Bacillus oligocarbophilus	$CO + \frac{1}{2}O_2 = CO_2$	74	66	(*b*)
Bacillus methanicus	$CH_4 + 2O_2 = CO_2 + 2H_2O$	212.6	194.6	(*b*)

[a] REFERENCES: (*a*) S. Winogradsky, *Ann. Inst. Pasteur* **4,** 213 (1890); (*b*) L. G. M. Baas-Becking and G. S. Parks, *Physiol. Revs.* **7,** 85 (1927); (*c*) R. L. Starkey, *J. Gen. Physiol.* **18,** 325 (1935); (*d*) M. W. Beijerinck, *Koninkl. Ned. Akad. Wetenschap.* **22,** 899 (1920); (*e*) F. C. Happold and A. Key, *Biochem. J.* **31,** 1323 (1937); (*f*) K. L. Temple and A. R. Colmer, *J. Bacteriol.* **62,** 605 (1951); (*g*) R. Wurmser, "Oxydations et Réductions." Presses Univ. France, Paris, 1930; (*h*) K. R. Butlin, M. E. Adams, and M. Thomas, *J. Gen. Microbiol.* **3,** 46 (1949); (*i*) J. R. Postgate, *J. Gen. Microbiol.* **14,** 545 (1956).

much energy on a molar basis as the oxidation of carbohydrates. For the complete aerobic oxidation of glucose to carbon dioxide,

$$C_6H_{12}O_6 + 6O_2 = 6CO_2 + 6H_2O$$

the heat of combustion and the free energy change, respectively, are $-\Delta H_{298^\circ} = +673$ kcal. and $-\Delta F^\circ_{298^\circ} = +694$ kcal. per mole of substrate.

2. *Utilization of Energy for Biosynthesis: Free Energy Efficiency*

a. Chemo-lithotrophic Bacteria. The chemo-lithotrophic way of life is characterized by the twofold ability to utilize litho-oxidations as the energy source and CO_2 as the sole carbon source; from this starting material the organism, which can develop in a purely inorganic medium, carries out biosyntheses of all cell constituents. Carbon dioxide fixation and assimilation is an endergonic process which requires at the same time free energy and reducing systems, as shown schematically by the equation

$$CO_2 + 4(H) + \text{free energy} = (CH_2O) + 2\ H_2O$$

In this expression, where (H) indicates reducing capacity of any origin, (CH_2O) represents the organic matter which has been synthesized and corresponds to the fact that carbon in cell constituents is, on the average, at the same degree of oxidation as it is in carbohydrates. This classical representation obviously is approximative; according to van Niel (9), the average degree of oxidation of the carbon could be more exactly indicated by (C_2H_3O), at least in the photosynthetic bacteria of the family of Thiorhodaceae. From the point of view of energetics, however, this comparison of the cell constituents with the sugars is in satisfactory agreement with the experimental results. Baas-Becking and Parks (*10*) measured the heat of combustion of several chemo-lithotrophic bacteria and found, on the average, 112 kcal. per gram-atom of carbon; this value is practically identical with the heat of combustion of glucose calculated on the same basis, 113 kcal. Similarly, Terroine and Wurmser (*11*) found 3480 cal. per gram of mycelium in the heterotrophic mold *Sterigmatocystis nigra*, in close agreement with the heat of combustion of a gram of glucose, 3700 cal. The amount of energy incorporated in the cellular matter by chemoautotrophic bacteria may therefore be set at 113 kcal. per gram-atom of carbon assimilated. From this value and the metabolic balance sheets of the cultures, the energy efficiency can be calculated as follows:

$$\text{Energy efficiency } (\%) = 100 \times \frac{\text{Carbon atoms assimilated} \times 113}{\text{Energy (kcal.) from amount of substrate oxidized}}$$

The first experimental approach along these lines was carried out by Winogradsky (*12*) who measured the N/C ratio (nitrogen oxidized and carbon assimilated) for cultures of nitrifying bacteria and found an average value of 35 for *Nitrosomonas* and of 135 for *Nitrobacter*. Under the most favorable culture conditions, Meyerhof (*13*) later obtained a value of 101 for the N/C ratio of *Nitrobacter*. These figures show that the assimilation of a carbon atom corresponds to the oxidation by *Nitrobacter* of 87 molecules of nitrite to nitrate. From the heat of combustion of nitrite, Meyerhof thus calculated the energy efficiency of *Nitrobacter* and arrived at the remarkably low figure of 4.5%.

Baas-Becking and Parks (*10*) reopened this question by considering not the heats of combustion but the free energy change during the oxidation of nitrogen-containing substrates under the conditions of the bacterial cultures:

Nitrosomonas spp.

$$NH_4^+(5 \times 10^{-3}\,M) + 3/2\,O_2 = NO_2^- + H_2O + 2H^+(10^{-8}) \qquad (-\Delta F_{298^\circ} = 66.5 \text{ kcal.})$$

Nitrobacter spp.

$$NO_2^-(7 \times 10^{-3} M) + 1/2\,O_2 = NO_3^- \qquad (-\Delta F_{298^\circ} = 17.5 \text{ kcal.})$$

Starting with the metabolic balance sheets of Winogradsky and of Meyerhof, then, the *free energy efficiency* of *Nitrosomonas* is 5.9% and that of *Nitrobacter* is 7.8%. Baas-Becking and Parks also calculated the free energy efficiencies of several other chemo-lithotrophic bacteria by this method. The results (Table IV) show values between 5 and 9%, except for *Hydrogenomonas* with a 30% efficiency; these values are much lower than those of heterotrophic bacteria which reach or even surpass efficiencies of 60%. This difference in total efficiency does not necessarily indicate that the energy-transport systems which connect the sequence of catabolic reactions with that of biosynthetic reactions are different or less efficient in autotrophs than in heterotrophs. Recent studies on the mechanism of carbon dioxide assimilation have shown, as will be discussed in Section I,C, that energy is transferred by the same type of phosphorylated compounds in the two types of microorganisms.

The manner in which the heterotrophic bacteria fix and assimilate carbon is not yet known precisely and may actually vary from one substrate or organism to the next. As a first approximation, it is likely that the starting point of the biosyntheses is a compound in which the carbon is in an oxidation state corresponding to (CHO), that is, intermediate between that of CO_2 and of the sugars and analogous to that of pyruvate. It is obvious that the biosynthesis of cell constituents from a carbon compound of this type requires fewer transfers and hence fewer energy losses, even if each stage in the reduction of carbon has

TABLE IV

FREE-ENERGY EFFICIENCY OF SOME CHEMO-LITHOTROPHIC BACTERIA[a]

Organism	Reaction	Free-energy efficiency (%)	References[b]
Hydrogenomonas spp.	$H_2 + \frac{1}{2}O_2 = H_2O$	26 4	(*a*)
Methanomonas spp.	$CH_4 + 2O_2 = CO_2 + 2H_2O$	0 6 29 6 (limits)	(*b*)
Nitrosomonas spp.	$NH_4^+ + \frac{3}{2}O_2 = NO_2^- + H_2O + 2H^+$	5 9	(*c*)
Nitrobacter spp.	$NO_2^- + \frac{1}{2}O_2 = NO_3^-$	7 9	(*c*)
Thiobacillus thiooxidans	$S + \frac{3}{2}O_2 + H_2O = H_2SO_4$	6 2	(*d*)
T. denitrificans	$6KNO_3 + 5S + 2CaCO_3 = 3K_2SO_4 + 2CaSO_4 + 2CO_2 + 3N_2$	5 0	(*e*)
T. denitrificans	$5Na_2S_2O_3 + 8KNO_3 + 2NaHCO_3 = 6Na_2SO_4 + 4K_2SO_4 + 4N_2 + 2CO_2 + H_2O$	8 7	(*e*)

[a] From L. B. Baas-Becking and G. S. Parks, *Physiol. Revs.* **7**, 85 (1927).

[b] REFERENCES: (*a*) W. Ruhland, *Jahrb. wiss. Botan.* **63**, 321 (1924); (*b*) N. L. Söhngen, *Rec. trav. chim.* **29**, 238 (1910); (*c*) L. Baas-Becking and G. S. Parks, *Physiol. Revs.* **7**, 85 (1927); (*d*) S. A. Waksman and R. L. Starkey, *J. Gen. Physiol.* **5**, 285 (1922); (*e*) R. Lieske, *Ber. Botin. Ges.* **36**, 12 (1912).

same unit efficiency for the two biosynthetic systems. The poor energy efficiency of the autotrophs can certainly be explained in large measure by this consideration.

Moreover, it is worth emphasizing, as was done by Lees (*14*), that free energy efficiencies calculated by Baas-Becking and Parks correspond on the whole to metabolic balance sheets derived from old cultures incubated for several weeks. Under these conditions the endogenous respiration of the bacteria is quite an appreciable factor, and the quantity of carbon found in cell constituents at the end of the incubation is the difference between the quantity of carbon dioxide actually

TABLE V

FREE-ENERGY EFFICIENCY OF CHEMO-LITHOTROPHIC BACTERIA IN SHORT-TERM EXPERIMENTS

Organism	Free-energy efficiency	References[a]
obacillus thiooxidans	approx. 50	(*a*)
bic Thiobacilli	maximum 16	(*b*)
denitrificans	maximum 25	(*b*)
thiocyanooxidans	25	(*c*)
ferrooxidans	3	(*d*)
drogenomonas facilis	30	(*e*)
omonas spp. "old" cultures	7	(*f*)
omonas spp. "young" cultures	maximum 20	(*f*)

[a] REFERENCES: (*a*) K. G. Vogler, *J. Gen. Physiol.* **26**, 103 (1942); (*b*) K. Baalsrud and S. Baalsrud, in "Phosphorus Metabolism" (W. D. McElroy and B. Glass, eds.), Vol. 2, p. 544. Johns Hopkins, Baltimore, 1952; (*c*) J. B. Yenzan, *J. Gen. Microbiol.* **11**, 1 (1954); (*d*) K. L. Temple and A. R. Colmer, *J. Bacteriol.* **62**, 605 (1951); (*e*) A. Mitz, *J. Microbiol.* **6**, 329 (1952); (*f*) T. Hofman and H. Lees, *Biochem. J.* **52**, 140 (1952).

id and that which is lost secondarily by endogenous respiration. To uce the influence of endogenous respiration to a minimum, Lees (*14*) l other authors used short-term cultures in Warburg flasks and ained much higher free energy efficiencies (Table V). A noteorthy exception is *Thiobacillus ferrooxidans* whose energy efficiency mains quite low even in short-term cultures, failing to rise above 3%. cording to Lees (*14*), this behavior has a double cause. On the one nd, the source of energy used by the organisms, i.e., the oxidation of rous to ferric iron, is a weakly exergonic reaction.

$$Fe^{2+} \rightarrow Fe^{3+} + e + 11.3 \text{ kcal.}$$

ich could provide the cell with only one high-energy bond per elecn, even if phosphorylation were assumed to have a 100% efficiency. the other hand, *T. ferrooxidans* develops in an extremely acid medium

(pH 2.0) and a large proportion of the energy furnished by the substrate may possibly be used to regulate the endocellular pH.

Lees (*14*) postulates that in other chemo-lithotrophic bacteria the energy efficiency of growth may also be reduced by the necessity of regulating the composition of the endocellular milieu. For example, nitrite produced by *Nitrosomonas* from ammonium ions is toxic for this organism and is possibly excreted by an active transport system at a considerable expenditure of energy.

b. Photo-lithotrophic Bacteria. This group includes the purple sulfur bacteria or *Thiorhodaceae* and the green sulfur bacteria or *Chlorobacteriaceae*. These organisms possess a chlorophyll which is chemically very similar to plant chlorophylls and they develop anaerobically in a purely inorganic medium, where CO_2 is the sole carbon source, if light and an exogenous electron donor are provided. The typical donors are sulfide and, for certain species, thiosulfate or tetrathionate, which are oxidized to elementary sulfur and sulfate. The sulfur compounds may be replaced by molecular hydrogen which is oxidized to water, and the purple sulfur bacteria can also utilize various organic compounds such as mono- or dicarboxylic acids as electron donors.

The nature of the source of energy used by these bacteria to assimilate carbon dioxide and carry out their biosyntheses has been the subject of a lengthy and interesting controversy. Winogradsky (*15*), who discovered them in 1888, considered these organisms as chemo-lithotrophs using for growth the chemical energy produced by the aerobic oxidation of sulfide to sulfate. Later, it was recognized that they are photosynthetic organisms related to the purple nonsulfur bacteria or *Athiorhodaceae* which Engelmann (*16*) had discovered in 1882; however, their physiology and energy metabolism remained obscure until 1931, when van Niel (*17*) began his masterful series of investigations on this subject. On the basis of a large body of experimental evidence, van Niel was led to postulate that the fundamental reaction of photosynthesis, in green plants as in bacteria, consists of the photolysis of water catalyzed by chlorophyll, with the formation of a reducing constituent (H) which the organism uses to hydrogenate CO_2, and of an oxidizing constituent (OH). In green plants the (OH) constituent is finally eliminated as free oxygen. The important finding that the oxygen formed during chlorophyll-mediated assimilation in green plants is derived from water and not, as believed earlier, from CO_2 was confirmed experimentally some years later by Calvin and his school with water labeled with heavy oxygen.

Moreover, van Niel showed that the green and purple sulfur bacteria are strict anaerobes and that, contrary to Winogradsky's conclu-

sions, they cannot oxidize mineral sulfur compounds aerobically in the presence of oxygen. He also demonstrated that, in contrast to plants, the photosynthetic bacteria cannot eliminate as O_2 the oxidizing constituent produced from the photochemical reaction, and that they must replace this mechanism by the oxidation of an exogenous electron donor. This difference between the photosynthesis of green plants and that of bacteria is shown schematically in Fig. 1.

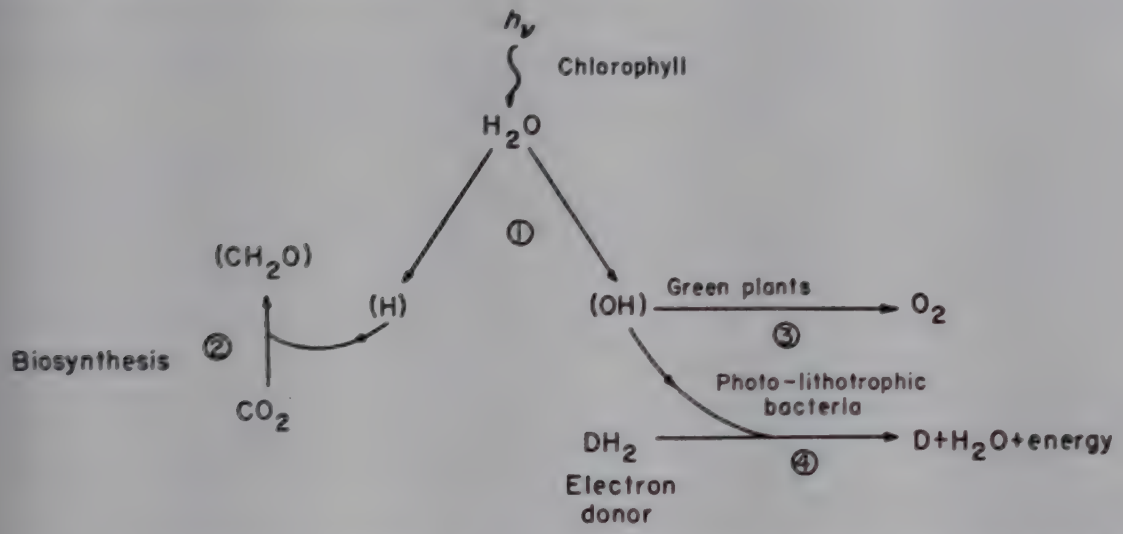


FIG. 1. Comparative scheme of photosynthesis in green plants and in photo-lithotrophic bacteria according to van Niel's theory.

If photosynthesis is considered from the thermodynamic point of view, it may be seen that in green plants the energy required for the assimilation of carbon dioxide

$$CO_2 + H_2O \rightarrow (CH_2O) + O_2 \qquad (-\Delta F^\circ_{298^\circ} = -115 \text{ kcal.})$$

can be provided in theory by 3 quanta of red light per molecule of CO_2. The experimental attempts to establish the amount of energy which is actually necessary led to discordant results and are still the subject of controversy. However, the most commonly accepted average value is about 9 quanta of light energy per molecule of fixed CO_2 (*18*).

In photo-lithotrophic bacteria, the oxidation of the exogenous electron donor constitutes an appreciable source of free energy. The question arises whether it may be utilized by the organism for biosynthetic activities. Following the work of Buder (*19*) and Blum (*20*), this question was first answered in the affirmative and the green and purple sulfur bacteria were considered to be both photosynthetic and chemo-lithotrophic. According to this interpretation, the energy balance sheet of the fixation of CO_2 by the sulfur bacteria is established in the presence of sulfide as follows:

$$CO_2 + 2H_2S \rightarrow (CH_2O) + 2S + H_2O \qquad (-\Delta F^\circ_{298^\circ} = -15 \text{ kcal.})$$

and the minimum amount of light energy which, theoretically, is necessary for the reaction to proceed is only one quantum per molecule of CO_2 assimilated, i.e., a third of the amount required for the assimilation of CO_2 in green plants. Larsen (*18*) measured the quantum yield of light during photosynthesis by a green sulfur bacterium (*Chlorobium thiosulfatophilum*) and a purple sulfur bacterium (*Chromatium* sp.) in the presence of different electron donors. The results (Table VI) show that

TABLE VI

THEORETICAL AND OBSERVED QUANTUM REQUIREMENTS OF VARIOUS BACTERIAL PHOTOSYNTHESES[a]

Organism	Electron donor	Oxidized form of electron donor	$-\Delta F°_{298°}$/mole of CO_2 (kcal.)	Number of quanta needed for assimilation of 1 mole CO_2	
				Theoretical minimum[b]	Observed minimum
Chromatium sp.	H_2	H_2O	1.7	0.05	9
	$S_2O_3^{2-}$	$S_4O_6^{2-}$	98.5	3.0	9
Chlorobium thiosulfatophilum	H_2	H_2O	1.7	0.04	9
	$S_2O_3^{2-}$	SO_4^{2-}	29.2	0.75	9
	$S_4O_6^{2-}$	SO_4^{2-}	30.2	0.77	9

[a] From H. Larsen, *4th Symposium Soc. Gen. Microbiol.* p. 186 (1954).

[b] For *Chromatium*, calculated for light of wavelength 870 mμ; for *Chlorobium*, calculated for light of wavelength 730 mμ.

whatever the thermodynamic balance sheet of the litho-oxidation coupled with photosynthesis, the quantity of light energy needed for the assimilation of CO_2 is constant and equal (9 quanta) to that of photosynthesis in green plants. These very important observations show that the energy produced by the chemical oxidation of the electron donor is not utilized for biosynthesis. In other words, they lead to the conclusion that the chlorophyll-containing bacteria, like the higher plants, are exclusively phototrophic.

C. Mechanism of Carbon Dioxide Assimilation by Chemo-lithotrophic Bacteria

The ability to metabolize CO_2 is not an exclusive property of chemolithotrophic bacteria and photosynthetic organisms. Other organisms and particularly the heterotrophic bacteria carry out numerous metabolic processes involving CO_2 in which this compound is reduced or incorporated in certain cell constituents. In the first group of these reactions, carbon dioxide is reduced directly to another C_1 compound. Thus,

Escherichia coli and other members of the colon-aerogenes group possess a hydrogenlyase system, one of whose constituents is the enzyme hydrogenase (cf. Section IV,A,3). This system carries out the reversible synthesis of formate from CO_2 and H_2:

$$CO_2 + H_2 \rightleftharpoons HCOOH$$

The strict anaerobe *Methanobacterium omelianskii* reduces CO_2 to methane by a process which was studied in detail by Barker *et al.* (*21*); carbon dioxide acts as the final hydrogen acceptor for the anaerobic oxidation of various organic substrates (DH_2):

$$CO_2 + 4DH_2 \rightarrow CH_4 + 4D + 2H_2O$$

It is noteworthy that these processes differ with those in which CO_2 is reduced by autotrophic organisms in that their physiological significance is purely catabolic. Barker showed that in *M. omelianskii* carbon dioxide is reduced to methane without being incorporated even transitorily in more complex carbon compounds. With regard to hydrogenlyase, it is also unlikely that the C_1 compound produced in this system can be incorporated into cell constituents and serve as the starting point of biosyntheses. Woods and Lascelles (*22*) stressed that the reaction in which formate is synthesized from CO_2 and H_2 is not very exergonic and cannot provide much free energy for biosynthesis from this starting point nor for autotrophic development of the organism under anaerobiosis. It seems that in heterotrophic bacteria the hydrogenlyase system operates physiologically in reverse, enabling the organism to oxidize formate anaerobically without the intervention of any exogenous hydrogen acceptor.

Furthermore, heterotrophic bacteria, yeasts, and higher animals possess biochemical processes in which carbon dioxide is incorporated into cell constituents by carboxylation of a C_2–C_5 compound. These reactions have been studied extensively in the last few years by Utter and Wood (*23*), Werkman (*24*), and Ochoa (*25*). Table VII lists some examples; from the point of view of comparative biochemistry, the most interesting are the modes of formation of oxalacetate by β-carboxylation of a C_3-acid. One of these reactions is the carboxylation of pyruvate by the malic enzyme of Ochoa *et al.* (*26*):

$$CO_2 + CH_3 \cdot CO \cdot COOH \rightleftharpoons COOH \cdot CH_2 \cdot CO \cdot COOH$$

This enzyme, which is found in *Lactobacillus arabinosus* and in pigeon liver, takes its name from the fact that in the presence of Coenzyme I (diphosphopyridine nucleotide, DPN) it also catalyzes the formation of malate from lactate:

$$CO_2 + CH_3 \cdot CHOH \cdot COOH \overset{+DPN}{\rightleftharpoons} COOH \cdot CH_2 \cdot CHOH \cdot COOH$$

TABLE VII

OVER-ALL REACTIONS FOR HETEROTROPHIC ASSIMILATION OF CARBON DIOXIDE BY BACTERIA[a]

Type of reaction	Over-all reaction	Experimental system	References[b]
$C_2 + C_1$	(1) $H_2 + CO_2 \rightleftharpoons$ Formate + Acetate $\rightleftharpoons$ Pyruvate	*E. coli* (cells)	(*a*)
	(2) $H_2 + CO_2$ + Acetate $\rightleftharpoons$ Pyruvate	*C. butylicum* (extracts)	(*a*)
$C_3 + C_1$	(1) Pyruvate + $CO_2 \rightleftharpoons$ Oxalacetate	*M. lysodeikticus* (extracts); *E. coli* and *P. morganii* (extracts)	(*a*) (*b*)
	(2) Pyruvate + CO_2 + $DPN_{,red.} \rightleftharpoons$ Malate + $DPN_{ox.}$	*L. arabinosus* (extracts)	(*a*)
	(3) Propionate + $CO_2 \rightleftharpoons$ Succinate	*P. pentosaceum* (cells)	(*c*)
$C_4 + C_1$	Succinate + $CO_2 \rightleftharpoons$ α-Ketoglutarate	*E. coli* (extracts)	(*a*)
$C_5 + C_1$	α-Ketoglutarate + CO_2 + $TPN_{,red.} \rightleftharpoons$ Isocitrate + $TPN_{ox.}$	*E. freundii* (extracts)	(*d*)

[a] From D. D. Woods and J. Lascelles, *4th Symposium Soc. Gen. Microbiol.* p. 1 (1954).

[b] REFERENCES: (*a*) M. F. Utter and H. G. Wood, *Advances in Enzymol.* **12,** 41 (1951); (*b*) J. P. Kaltenbach and G. Kalnitsky, *J. Biol. Chem.* **192,** 629, 641 (1951); (*c*) S. Barban and S. J. Ajl, *J. Biol. Chem.* **192,** 63 (1951); (*d*) S. Barban and S. J. Ajl, *J. Bacteriol.* **66,** 68 (1953).

The second reaction leading to the formation of oxaloacetate is the carboxylation of phosphoenolpyruvate by the oxaloacetate-synthesizing enzyme of Utter and co-workers (*27*):

$$CO_2 + CH_2{=}C(O{-}PO_3H_2){-}COOH + \text{Inosine diphosphate} \rightleftharpoons COOH \cdot CH_2 \cdot CO \cdot COOH + \text{Inosine triphosphate}$$

This enzyme is distributed widely. First shown to occur in the cells of *Micrococcus lysodeikticus* and in pigeon liver, it was later found in other bacteria.

Direct carboxylation of phosphoenol pyruvate, without the participation of nucleotide, is performed by phosphopyruvate carboxylase, which was found by Pomerantz (*28*) in *Propionibacterium shermanii* and recently demonstrated (cf. below) in chemo-lithotrophic bacteria.

Little is known at this time about the significance of these processes for the general metabolism of heterotrophic organisms. It has long been recognized that certain bacteria can develop only in the presence of free CO_2 and it is likely that at least in these organisms these carboxylations play an essential physiological role; but it does not appear that heterotrophs can incorporate appreciable quantities of carbon by this pathway (*22*).

Recently, Aubert and associates (*29*) applied to the problem of autotrophy the radiochemical techniques used by Calvin and his school to elucidate the mechanism of photosynthesis in plants. They found that the first stable compound into which the radioactivity of $C^{14}O_2$ is incorporated by cells of *Thiobacillus denitrificans* is 3-phosphoglyceric acid; this is formed from ribulose diphosphate by the reaction which in chlorophyll-containing plants is catalyzed by carboxydismutase.* The nature of the compounds formed later, their kinetic order of appearance, and their isotopic labeling pattern indicate that ribulose diphosphate is resynthesized from 3-phosphoglyceric acid by way of a cycle identical to that described by Calvin in plants.

The various enzymes of the Calvin cycle were then demonstrated in extracts of this bacterium by Trudinger (*30*). Bergmann *et al.* (*31*) and McFadden (*32*) also showed that the Calvin cycle is present in *Hydrogenomonas*, and these findings indicate that this mechanism of carbon dioxide assimilation probably is a very general characteristic of chemolithotrophic bacteria.

In the course of their work with *T. denitrificans*, Aubert and coworkers demonstrated on the basis of the radioactive labeling pattern of glutamic and aspartic acids that phosphoenolpyruvate is carboxylated

* This enzyme is also known as diphosphoribulose carboxylase.

by this organism to yield oxaloacetate by an enzymatic process identical with the Pomerantz reaction (28). Therefore, a double mechanism is involved in the fixation of CO_2 (Fig. 2). One molecule of CO_2 is fixed on ribulose diphosphate by a reaction similar to that which occurs in photosynthesis, and a second by carboxylation of phosphoenolpyru-

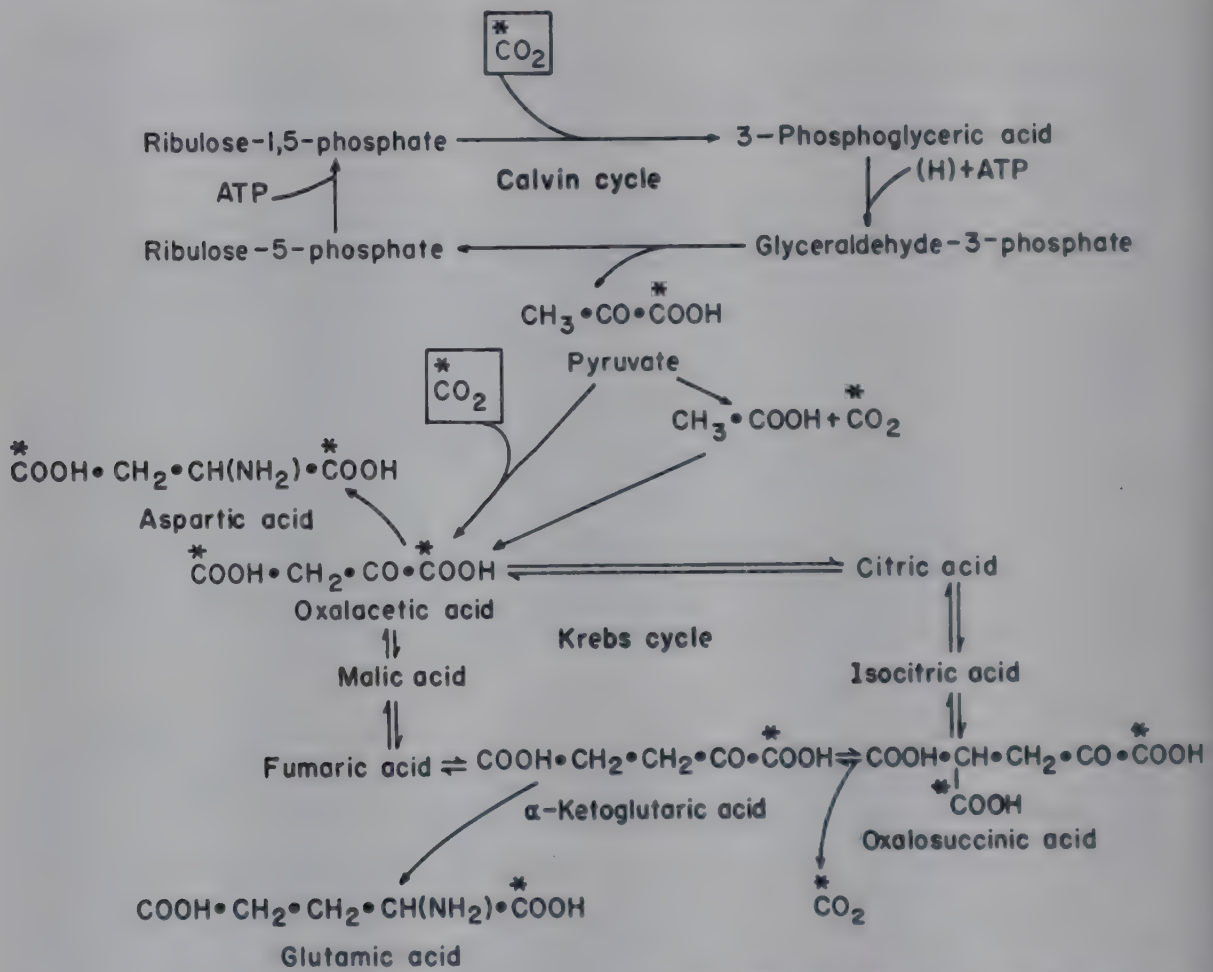


FIG. 2. Mechanism of $C^{14}O_2$ incorporation by *Thiobacillus denitrificans* cells as shown by Aubert *et al.* (29). One molecule of carbon dioxide is fixed on ribulose-1,5-phosphate (Calvin cycle) and a second by carboxylation of phosphoenolpyruvate. The aspartic acid is formed from oxalacetate and labeled in both the C-1 and C-4 positions. The glutamic acid is formed through the Krebs cycle intermediates from α-ketoglutarate and labeled in the C_1 position only. The labeled carbons are marked with an asterisk. The symbols ATP and (H) show the steps of the primary Calvin cycle where phosphorylation and reduction are involved respectively.

vate, i.e., by a reaction analogous to that carried out by *P. shermanii* and higher animals. Determinations of the relative importance of the two modes of incorporation indicated that under these experimental conditions 90% of the total CO_2 is fixed by the Calvin cycle and the remainder by the second reaction.

The coexistence of the Calvin cycle and of other carboxylation systems appears to be a general fact in chemo-lithotrophic bacteria. Judis *et al.* (33) obtained extracts from *Hydrogenomonas facilis* which fix

$C^{14}O_2$ in the presence of pyruvate or, if pyridine nucleotides are available, of malate. The same extracts can also catalyze the carboxylation of α-ketoglutarate to yield isocitrate. Bergmann *et al.* (*31*) confirmed these results and showed that *H. facilis* can carry out β-carboxylation of pyruvate or phosphoenolpyruvate in addition to fixing CO_2 on ribulose diphosphate. Finally, Suzuki and Werkman (*34*) found that cell-free extracts of *T. thiooxidans* contained at the same time the enzymes of the Calvin cycle and phosphoenolpyruvate carboxylase.

The mechanisms by which chemo-lithotrophic bacteria couple their litho-oxidative and biosynthetic reactions are little understood. Vogler and Umbreit (35) studied this problem in *T. thiooxidans,* which oxidizes elementary sulfur or thiosulfate to sulfuric acid aerobically. They found that in the presence of sulfur and oxygen and in the absence of CO_2 from the medium, nonproliferating suspensions of these bacteria actively incorporate inorganic phosphate. If the cells are then deprived of sulfur and oxygen and placed in the presence of carbon dioxide, they fix CO_2 and liberate inorganic phosphate. These findings were interpreted to mean that the bacterial cells can place into reserve as high-energy phosphate bonds the energy produced by the oxidation of sulfur and can then use this energy to fix CO_2. Baalsrud and Baalsrud (*36*) took up this study with the same organism but changed the substrate from elementary sulfur to thiosulfate. They confirmed that under these conditions the oxidation of the substrate is accompanied by a slight decrease in the content of inorganic phosphate in the medium and thus, most likely, by phosphorylation. But these authors, and later Newburg (*37*), did not find any appreciable CO_2 fixation in the absence of substrate, thus failing to confirm the observations and interpretation of Vogler and Umbreit (35).

These results, obtained before the mechanism of CO_2 incorporation has been clarified, lend considerable weight to the hypothesis that the coupling of the litho-oxidative and biosynthetic pathways takes place by means of energy-rich phosphate bonds. The demonstration of a Calvin cycle in these organisms makes it possible to determine the nature and number of the reactions into which phosphate esters enter. According to the Embden-Meyerhof scheme, the reduction to 3-phosphoglyceraldehyde of two molecules of 3-phosphoglyceric acid formed by the fixation of one molecule of CO_2 to ribulose diphosphate requires two molecules of adenosine triphosphate (ATP) and two molecules of reduced diphosphopyridine nucleotide (DPNH + H^+):

$$\text{3-Phosphoglyceric acid} + \text{ATP} \rightarrow \text{1,3-Diphosphoglyceric acid} + \text{ADP}$$

$$\text{1,3-Diphosphoglyceric acid} + \text{DPNH} + H^+ \rightarrow \text{3-Phosphoglyceraldehyde} + \text{DPN}^+ + P_i$$

A third molecule of ATP is consumed in the phosphorylation of ribulose-5-phosphate to ribulose-1,5-diphosphate.

Aubert *et al.* (29) showed that in *T. denitrificans* the synthesis of ribulose diphosphate from ribulose monophosphate requires ATP, and that the oxidation of thiosulfate is coupled to the formation of ATP. The mechanism by which ATP is produced is unknown. Recent experiments of Vishniac and Santer (38) and Skarzynski and Ostrowski (39) showed that during the oxidation of thiosulfate by *T. thioparus* intermediate compounds appear which contain both sulfur and phosphorus but whose chemical nature is not yet known.

The transfer of electrons from the litho-oxidative reaction to the biosynthetic pathway is probably mediated by a complex system involving cytochromes. As discussed below, several authors have demonstrated that thiobacilli contain cytochromes, some of which are of the type c, which are reduced by thiosulfate in the presence of cell-free bacterial extracts. The electrons needed for the reduction of 3-phosphoglyceric acid are doubtless transferred from these cytochromes to the substrate by the pyridine nucleotides.

Although information is not yet available on the mechanism of CO_2 fixation in other autotrophic bacteria and particularly in nitrifying bacteria, it is likely that these organisms behave in a manner analogous or identical to the thiobacilli and the hydrogen bacteria. The chemo-lithotrophic bacteria thus seem to be *bridge organisms* in which the biosynthetic systems of chlorophyll-containing plants and of higher animals are united. These facts are of great interest in comparative biochemistry since they demonstrate the remarkable similarity in the processes of intermediary carbon metabolism and of energy transport systems found in all living beings.

D. Interrelations between the Chemo-lithotrophic and Heterotrophic Modes of Nutrition

The chemo-lithotrophic bacteria fall into two groups with regard to their behavior toward exogenous organic matter. The first group, comprising only the nitrifying bacteria and most of the thiobacilli, is that of the *strict* or *obligatory autotrophs*, i.e., organisms incapable of using any form of organic matter as either the source of energy or of carbon. The second group, featuring particularly the hydrogen bacteria, is that of the *facultative autotrophs*, which can grow either autotrophically on a purely inorganic medium or heterotrophically on various organic compounds.

Winogradsky and Omeliansky (40) observed that nitrifying bacteria not only are unable to utilize organic substrates but are actually inhibited by low concentrations of glucose, peptone, asparagine, or so-

dium butyrate and by higher concentrations of urine, broth, or sodium acetate. This inhibitory effect of organic substances gave rise to the classical postulate of a sort of antinomy between the autotrophic and heterotrophic systems. However, van Niel (*3*) stated that the importance of these facts had been greatly exaggerated. Meyerhof (*41*) had observed earlier that *Nitrosomonas* and *Nitrobacter*, though inhibited by amino acids and amines, tolerated relatively high concentrations of organic nitrogen-free compounds, particularly sugars. Jensen (*42*) confirmed that pure cultures of *Nitrosomonas* are not inhibited by 0.3–0.6 *M* concentrations of organic acids, polyalcohols, and carbohydrates and established that the inhibition by glucose reported by earlier authors was in reality caused by toxic decomposition products formed during heat sterilization of the sugar. According to Kingma-Boltjes (*43*) and others (*44–46*), growth of *Nitrosomonas* is even enhanced by certain organic compounds, especially carbohydrates; this would explain the well-known fact that nitrification is more rapid on natural soils rich in organic matter than on poor soils or in artificial culture on purely inorganic media.

Lees (*47*) attributes the inhibition of growth in nitrifying bacteria by low concentrations of amino acids to the formation in the culture medium of chelates between the amino acids and certain oligo-elements indispensable for the growth of the organisms. Nitrogen compounds may also inhibit growth by other mechanisms. Thus methionine, which inhibits nitrification strongly in soil (*48*) and in artificial cultures during growth (*49, 50*), may act indirectly by giving rise to —SH groups, which are very toxic to nitrifying bacteria.

With the exception of two facultative autotrophs, one identified as *Thiobacillus novellus* by Starkey (*51*) and the other described by Tyulpanova-Mosevich (*52*), the thiobacilli are obligatory autotrophs which cannot utilize any organic compound. This behavior cannot be explained by the lack of appropriate enzyme systems, since—as we have seen—these organisms and particularly *T. thioparus* and *T. denitrificans* possess the same enzymes for carbon metabolism as do the heterotrophic bacteria. The postulated absence of "permeases," i.e., of active systems which would provide for the endocellular penetration of organic substrates (*53*), constitutes a very reasonable hypothesis, but experimental verification has not yet been possible.

In fact, chemo-lithotrophic organisms are distinguished from heterotrophs not so much by any fundamental differences in their biochemical activities as by the remarkable extent of biosynthetic capability in chemo-lithotrophs, which enables them to produce from carbon dioxide, ammonia, and a few inorganic salts the entire range of their cellular constituents and the growth factors necessary for their development.

In this connection it is of interest that Rittenberg and Grady (*54*), by ultraviolet irradiation of a normal strain of *T. thiooxidans*, obtained auxotrophic mutants which had lost a part of their biosynthetic capabilities and which grew only on a medium supplemented with thiamine or yeast extract.

It has long been held that autotrophic and heterotrophic modes of nutrition cannot coexist in facultative autotrophs. This opinion was based on the observations of Niklevskii (*55*) and Ruhland (*56*) who found that the presence of an utilizable organic substrate in the culture medium completely suppresses the consumption of hydrogen by *Hydrogenomonas*. Kluyver and Manten (*57*) reported later that cells of *H. flava* grown on organic media are effectively devoid of hydrogenase, but that cells derived from cultures grown on inorganic media oxidize lactate and H_2 simultaneously and in an additive fashion, indicating that coexistence of the two modes of nutrition in the cell is indeed possible. More recently, Wilson *et al.* (*58*) showed that if cultures are maintained at a partial pressure of oxygen below 5%, the presence of metabolizable organic substrates in the medium exerts no inhibition on the formation of hydrogenase by cells of *H. facilis;* under these conditions, lactate and H_2 are consumed simultaneously.

II. Oxidation of Nitrogen Compounds

In theory, there ought to be numerous inorganic forms of nitrogen which can be oxidized in biological systems and which could thus serve as energy sources for growth of chemo-lithotrophic organisms. As pointed out by Baas-Becking and Parks (*10*), it might be expected *a priori* that there are organisms which can utilize for their growth the energy produced by the aerobic oxidation of N_2 to nitrate, or of NH_3 to N_2. But such biological systems have never been demonstrated and their existence is very unlikely since these oxidations are not very exergonic and, with regard to the oxidation of N_2, the required activation of the biatomic gaseous molecule would entail a considerable expenditure of energy (225 kcal./mole).

In fact, the only known type of nitrogen litho-oxidation is the process of nitrification, i.e., the aerobic oxidation of an ammonium ion to nitrite and nitrate.

A. Nitrifying Microorganisms

1. *Chemo-lithotrophic Nitrifying Bacteria*

The biological oxidation of ammonia in the soil was demonstrated by Schloesing and Muntz (*59*) in 1877. However, it was some years later, in 1890, that Winogradsky (*60*) isolated and described two of

the bacterial agents responsible for this phenomenon; one, *Nitrosomonas,* oxidizes ammonia to nitrite

$$NH_4^+ + 3/2O_2 = NO_2^- + H_2O + 2H^+ \qquad (-\Delta F^\circ_{298^\circ} = 66.5 \text{ kcal.})$$

while the other, *Nitrobacter,* oxidizes nitrite to nitrate

$$NO_2^- + 1/2O_2 = NO_3^- \qquad (-\Delta F^\circ_{298^\circ} = 17.5 \text{ kcal.})$$

Winogradsky established that these organisms depend entirely on the oxidation of their respective inorganic substrates for energy, and this discovery led him to formulate for the first time the concept of the chemo-autotrophic way of life. He also showed that the two nitrification reactions proceed successively in the soil or in enrichment cultures; the oxidation of nitrite to nitrate by *Nitrobacter* is inhibited by NH_4^+ and cannot begin until all the ammonia initially present has been oxidized to nitrate by *Nitrosomonas.*

"Bergey's Manual" (*61*) describes two species in the genus *Nitrosomonas* and four other genera of chemo-lithotrophic bacteria which oxidize nitrite to nitrate. Of the latter four, the existence of only one, *Nitrosococcus,* described by Winogradsky himself (*60*), has been unquestioned. The three others (*Nitrosospira, Nitrosocystis,* and *Nitrosogloea*) are of doubtful validity, according to several authors (*62*). Among the organisms which carry out the nitrite-nitrate step, the genus *Nitrobacter* includes a mobile species described and isolated by Nelson (*63*) in addition to the type species which is the immobile form discovered by Winogradsky. A second genus, *Nitrocystis,* brings together forms endowed with the same physiological activity but which are morphologically distinct because of the production of zoogloea.

The literature on the taxonomy and physiology of nitrifying bacteria was analyzed recently by Bisset and Grace (*62*) and Meiklejohn (*64*).

2. *Heterotrophic Nitrifying Microorganisms*

There are numerous heterotrophic bacteria which oxidize ammonium salts anaerobically to nitrite or nitrate but cannot utilize the resulting energy for growth. According to Cutler and Crump (*65*), 104 different species produce traces of nitrite from ammonium ions in their cultures. More recently, Quastel *et al.* (*66*) showed by their soil perfusion method that several heterotrophic bacteria which cannot oxidize NH_4^+ nevertheless form nitrite and nitrate from pyruvic oxime. In contrast to *Nitrobacter* and *Nitrosomonas,* these organisms are not inhibited by methionine, thiourea, or ethyl urethan and can even oxidize the latter compound with the formation of nitrate. Jensen (*67*) made similar observations with bacteria of the genera *Alcaligenes* and *Aerobacterium* and with the mold *Nocardia.*

Isenberg *et al.* (*68*) isolated an actinomycete which oxidizes NH_4^+ rapidly to nitrite and Schmidt (*69*) found that the mold *Aspergillus flavus* produces significant quantities of nitrite and nitrate in peptone broth cultures. The origin of the nitrate has not been clearly established. This may involve the mineralization of organic nitrogen in the form of ammonia, which might then be oxidized to nitrite and further to nitrate. But Eyler and Schmidt (*70*) demonstrated very recently that *A. flavus* produces free nitrite and nitrate only on media where the nitrogen source is proteinaceous, namely yeast extract, peptones, and casein. The organism develops well on ammonium sulfate but without producing nitrite or nitrate. These observations suggest that nitrite and nitrate produced in the cultures of certain heterotrophs are not formed by the oxidation of free ammonium ion but by way of organic compounds of as yet undefined nature.

The amounts of nitrate found in cultures of the most active heterotrophs do not exceed a few micrograms per milliliter and are much lower than those obtained in cultures of *Nitrobacter*. However, according to Fischer *et al.* (*71*), the heterotrophic nitrifiers in soil are so numerous that their activity may constitute an appreciable factor in the nitrogen cycle in nature.

B. Biochemistry of Nitrification

The biochemistry of nitrification has been analyzed recently by Lees (*14*), Quastel and Scholefield (*48*), Delwiche (*72*), and Nason and Takahashi (*73*). The extensive literature on this subject actually reveals how little is known about the metabolic pathways of this important process. The slow progress in this area can doubtless be attributed in large measure to the lack of techniques which would permit the cultivation of nitrifying bacteria in quantities sufficient for the application of current methods of bacterial biochemistry.

During the oxidation of ammonium ion to nitrate, the valence of the nitrogen atom changes from -3 to $+5$, corresponding to the loss of 8 electrons. Since biological oxidations generally involve the loss of one or two electrons, Klyuver and Donker (*74*) proposed a nitrification scheme, which has become classic, postulating four successive stages, with a loss of two electrons or hydrogen atoms in each:

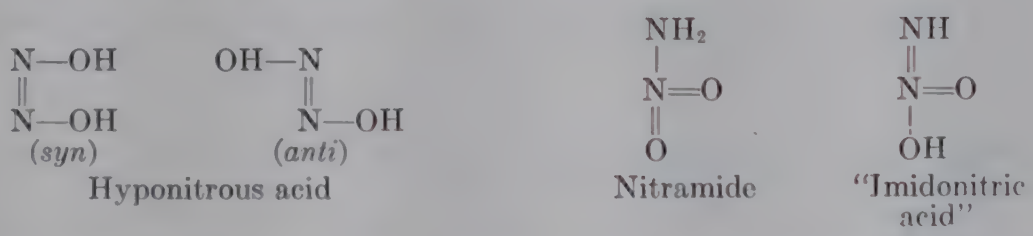
$$NH_4OH \xrightarrow[+\frac{1}{2}O_2]{(1)} NH_2OH \xrightarrow[+\frac{1}{2}O_2]{(2)} (NOH) \xrightarrow[+\frac{1}{2}O_2]{(3)} NO_2H \xrightarrow[+\frac{1}{2}O_2]{(4)} NO_3H$$

Nitrosomonas carries out the first three of these reactions and *Nitrobacter* the fourth.

The actual chemical nature of several of the intermediates featured

in this reaction sequence and the identity of the pertinent enzymes are still largely hypothetical. The intermediary formation of hydroxylamine (NH_2OH) has long been questioned in view of the pronounced toxicity of this compound for *Nitrosomonas*. But Lees (*75*) found that NH_2OH is oxidized by this organism as rapidly as NH_4^+, as long as its concentration remains below 1.5 mg. nitrogen per milliliter. Further experimental proof of the participation of NH_2OH in this sequence was furnished by Hofman and Lees (*76*) who found that NH_2OH accumulates in cultures of *Nitrosomonas* when the formation of nitrite is inhibited by hydrazine.

The next stage, where the degree of oxidation of nitrogen is represented by (NOH), is of very great importance for the metabolism of inorganic nitrogen in general. This is, in effect, the stage in the reduction of nitrate where the bifurcation occurs which divides the pathway leading to ammonia and that leading to gaseous nitrogen, i.e., to denitrification proper. Despite its importance, this stage is completely unknown at this time. Klyuver and Donker (*74*) have pointed out that the nitroxyl radical NOH is extremely unstable and must polymerize immediately to a more stable form. Several configurations have been postulated for such a dimer:



Among these hypothetical intermediates, hyponitrous acid has been suggested most frequently, however its formation is very unlikely. In fact, Hofman and Lees (*77*) tried without success to demonstrate oxidation of hyponitrite to nitrite by cells of *Nitrosomonas*. Further, Delwiche (*72*) stressed that hyponitrous acid is quite unstable at physiological pH and tends to decompose rapidly to N_2O and N_2 under these conditions. This property may favor a role of hyponitrous acid as an intermediate in denitrification since in this process one may find under certain experimental conditions that N_2O and N_2 are formed together. By contrast, it argues strongly against the hypothesis that hyponitrous acid may be an intermediate in the oxidation of hydroxylamine to nitrite during nitrification. The same considerations speak against the intermediary formation of nitramide, an even more unstable compound which is more rapidly decomposed to N_2O and N_2. Concerning the fourth structure postulated above, no definite demonstration of the chemical existence of "imidonitric" acid is at hand. All in all, it is

possible that the intermediary compound corresponding to the
of oxidation (NOH) is not formed in the free state but only as
zyme-substrate complex or in bound, organic form.

Few studies have been made and very little is known abo
enzymes which catalyze the oxidation of ammonia to nitrite. I
and Lees (78) showed that the well-known inhibition of Nitros
by allylthiourea takes place specifically in the first metabolic ste
during oxidation of ammonium ion to hydroxylamine, and do
affect the oxidation of NH_2OH to nitrite. They further reported t
enzyme system which catalyzes the first reaction requires the p
of a metal of unidentified nature. Recently, Imshenetskii et al. [7
pared extracts of cells of *Nitrosomonas europea* which oxidize a
and hydroxylamine to nitrite in vitro. The enzyme is relatively
stable, retaining its activity after brief exposure to 100° but w
maintenance of this temperature for 30 minutes.

Specific "reductases" have been extracted from various hetero
bacteria, molds, and higher plants; these enzymes catalyze re
which are the inverse of that carried out by *Nitrosomonas*, i.e.,
duction of nitrite and hydroxylamine to ammonia. Spencer et a
and Zucker and Nason (80) demonstrated that these enzym
metalloflavoproteins coupled functionally with pyridine-nucleo
pendent dehydrogenases. They are related to nitrate reducta
similarity is particularly striking with the nitrite reductase of
spora crassa (79), in which the metal has been shown to be ir
rum, as in nitratase. It may be thought a priori that the enzyme
enable *Nitrosomonas* to oxidize ammonia are reversible reduc
closely related enzymes. In support of this hypothesis, Klareve
Bard (81) made cell-free extracts of *Bacillus subtilis* containin
pobol dehydrogenase which catalyzed the reduction of NH_2OH
monia in the presence of ethanol, glucose-6-phosphate, or red
enzyme I (DPNH). They reported the reduction, by these prep
of oxidized diphosphopyridine nucleotide (DPN) in the pres
ammonia and considered the reversibility of the reaction as
However Bowers *et al.* (82) could not confirm this latter p
showed that the observed reduction of DPN by ammonia was
a nonenzymatic process without physiological significance.

The equilibrium constants of the reactions catalyzed by ni
hydroxylamine reductases (78, 80) favor the formation of ammo
the oxidation of pyridine nucleotides so strongly that their ph
ical function in the inverse direction, toward nitrification, is
likely. This is equally true of nitrate reductase; it is, therefore

to expect that the nitrification enzymes are entirely different in
from those involved in the reduction of nitrate to ammonia.
e oxidation of nitrite to nitrate by *Nitrobacter* most probably
place in a single enzymatic step, but the nature of this enzyme is
own. It has long been known that this reaction is inhibited by
te; recently, Lees and Simpson (83) demonstrated that this in-
e has an indirect effect. It is first transformed by the bacteria to
e which is the true specific inhibitor. These authors showed that
te of oxidation of nitrite is proportional to the content in Nitro-
cells of a cytochrome purified and studied by Burt and Lees
This is a cytochrome c with an absorption maximum at 551 mμ
takes on a functional role in the electron transport in the oxida-
f nitrite. Finally, Zavarzin (85) indicated that molybdenum and
ron take part in this reaction.

Oxidation of Sulfur and Sulfur Compounds

A. Sulfo-oxidizing Bacteria

Thiobacilli

e genus *Thiobacillus* comprises chemo-lithotrophic sulfo-oxidizing
ria lacking chlorophyll and intracellular sulfur granules. The prin-
species are shown in Table VIII.
orphologically, these bacteria are thin and short, measuring about
1 to 3 μ, nonsporeforming, Gram-negative, generally motile and,
e instances, equipped with a single polar flagellum. Very widely
ted in nature, the thiobacilli, like the morphologically quite
r *Pseudomonas*, are constant inhabitants of soil and fresh or salt

l the species of thiobacilli use for growth the energy provided by
idation of sulfide and of thiosulfate:

$$HS^- + 2O_2 = SO_4^{2-} \qquad (-\Delta F^\circ_{298} = +171 \text{ kcal.})$$
$$S_2O_3^{2-} + 2.5\,O_2 = 2SO_4^{2-} \qquad (-\Delta F^\circ_{298} = +237.6 \text{ kcal.})$$

veral species can also oxidize elementary sulfur, more or less
ly:

$$S^0 + 1.5\,O_2 + H_2O = SO_4^{2-} + 2H^+ \qquad (-\Delta F^\circ_{298} = +118.9 \text{ kcal.})$$

ese reactions, which result in the complete oxidation of sulfur and
rmation of free sulfuric acid, cause a considerable drop in the
f the cultures. The tolerance towards this acidity varies in bacteria
species to species. Thus *T. thioparus* develops best at near-neutral

pH and stops growing when the pH drops below 5.0 (*86*). At the other end of the scale there are strains of *T. thiooxidans* and *T. ferrooxidans* which grow only at pH levels below 5.0 and which can develop at zero or even at negative pH values (*87*). Many intermediate forms also exist.

TABLE VIII

THE PRINCIPAL MEMBERS OF THE BACTERIAL GENUS *Thiobacillus*

Organism	Optimum pH	Remarks	References[a]
T. thioparus	approx. 7.0	Type species	(*a*, *b*)
T. denitrificans	approx. 7.0	Facultative anaerobe; able to grow anaerobically on media containing nitrate which is reduced to N_2	(*b*)
T. thiooxidans	5.0	Elementary sulfur is rapidly oxidized; at the end of incubation, pH in the cultures can be lowered to 0 or even to negative values	(*c*)
T. ferrooxidans	5.0	Very similar to *T. thiooxidans*, with the difference that elementary S is slowly oxidized and that sulfur compounds can be replaced as energy source by the oxidation of ferrous to ferric iron	(*d*)
T. thiocyanooxidans	7.0	Thiocyanate can be utilized as sole source of energy, carbon, and nitrogen	(*e*)
T. novellus	7.0	Facultative autotroph; can grow on organic media in the absence of inorganic sulfur compounds	(*f*)

[a] REFERENCES: (*a*) A. Nathanson, *Mitt. zool. Station Neapel* **15,** 655 (1902); (*b*) M. W. Beijerinck, *Centr. Bakteriol.* **II/11,** 593 (1904); (*c*) S. A. Waksman and J. S. Joffe, *Science* **53,** 216 (1921); (*d*) A. R. Colmer, K. L. Temple, and M. E. Hinkle, *J. Bacteriol.* **59,** 317 (1950); (*e*) F. C. Happold and A. Key, *Biochem. J.* **31,** 1323 (1937); (*f*) R. L. Starkey, *Soil. Sci.* **39,** 197 (1935).

The thiobacilli are strict aerobes, with the single exception of the facultative anaerobe *T. denitrificans*. The metabolism of this species is identical with that of *T. thioparus* in aerobiosis, but it can also grow in the absence of free oxygen if the medium contains nitrate, which is reduced to gaseous nitrogen:

$$3S_2O_3^{2-} + 5NO_3^- = \tfrac{5}{2}N_2 + 6SO_4^{2-} \qquad (-\Delta F^\circ_{298^\circ} = +549 \text{ kcal.})$$

It should be emphasized that nitrate is utilized only for the oxidation of the sulfur-containing substrate and is not assimilated. For *T.*

denitrificans as for the other members of the genus, the sole source of assimilable nitrogen is ammonia, which is usually provided in the culture medium as ammonium chloride.

Among the species listed in Table VIII, three are distinguished by the fact that they can make use of other sources of energy than inorganic sulfur compounds. *T. novellus* is a facultative autotroph which can grow on organic media. *T. ferrooxidans* can use the oxidation of Fe^{2+} to Fe^{3+} as an energy source. Finally, *T. thiocyanooxidans* can use thiocyanate as the sole source of energy and of carbon and nitrogen. The metabolic systems characteristic of the latter two organisms are described in Sections V,A and V,B.

Several species of thiobacilli of doubtful validity have been reported. Parker (*88*) described a bacterium responsible for the corrosion of concrete in sewers, with the production of large quantities of free sulfuric acid from the sulfides present in sewage. This organism, which he called *T. concretivorus*, does not seem to differ morphologically or biochemically from *T. thiooxidans*.

Baalsrud (*89*) and Vishniac and Santer (*38*) recently reviewed the extensive literature on the thiobacilli, their taxonomy, methods of culture, and physiological properties.

2. *Beggiatoaceae and Achromaciaceae*

These two families of bacteria comprise nonphotosynthetic sulfo-oxidizing organisms which differ from the thiobacilli because they contain endocellular granules of elementary sulfur.

The family of Beggiatoaceae, to which "Bergey's Manual" (*61*) assigns 4 genera (*Beggiatoa, Thioploca, Thiothrix,* and *Thiospirillopsis*), is characterized by filamentous, unbranched, multicellular, alga-like cells. These organisms resemble the algae *Oscillatoria;* they share their very particular means of motion by reptation, but differ in lacking chlorophyll and phycocyanin. The family of Achromaciaceae, which consists of three genera (*Achromatium, Thiovulum,* and *Macromonas*), is made up of unicellular, nonfilamentous, mobile or immobile, nonpigmented organisms, some of which closely resemble the Myxophyceae.

The classification of these bacteria and their place in taxonomy are not yet established clearly and are the subject of controversies discussed by Bisset and Grace (*62*). Little is known about their metabolism although they were first studied in 1887. Winogradsky (*90*) showed that the oxidation of sulfide by *Beggiatoa alba* proceeds in two steps. The first is the aerobic oxidation to elementary sulfur which accumulates in the cell as strongly refringent granulations:

$$H_2S + 0.5\,O_2 = S^0 + H_2O + 41 \text{ kcal.}$$

When the sulfide initially present has disappeared from the medium, the bacteria carry out the oxidation of S^0 to sulfate:

$$S^0 + 1.5\,O_2 + H_2O = H_2SO_4 + 118 \text{ kcal.}$$

so that the intracellular sulfur granules may be considered to some extent as nutritive and energy-yielding reserves.

According to Winogradsky (*90*), *Beggiatoa* is an autotroph which uses sulfides and CO_2 as sole carbon and energy sources. This opinion is also held by Keil (*91*) but rejected by others. Certain experiments of Cataldi (*92*), in fact, suggest that at least some strains of *Beggiatoa* cannot grow in the complete absence of organic matter and are therefore heterotrophic organisms.

3. *Photosynthetic Sulfo-oxidizing Bacteria*

The essential physiological characteristics of these organisms, which include the purple sulfur bacteria (or Thiorhodaceae) and the green sulfur bacteria (or Chlorobacteriaceae) were described in Section I of this chapter. It was pointed out in particular that van Niel (*17*) and Larsen (*18*) showed that these organisms use only inorganic sulfur compounds as electron donors in their characteristic type of photosynthesis and that they cannot utilize the free energy produced by the oxidation of their inorganic substrates for biosynthetic reactions.

Each of the two families, the Thiorhodaceae and the Chlorobacteriaceae, comprise several genera and species whose morphological characteristics and taxonomic relationships were analyzed by Bisset and Grace (*62*). Most of the types which have been described are not well known and in some instances it is even doubtful whether they exist at all. Van Niel (*17*) showed that several forms thought to be distinct species are in reality interconvertible if the developmental conditions are modified and actually belong to a restricted number of different types.

Almost everything known about the metabolism of the photosynthetic sulfur bacteria concerns the genus *Chromatium* among the purple bacteria and the genus *Chlorobium* among the green bacteria. Larsen (*93*) isolated and described two species of the latter which were identical morphologically but behaved differently toward inorganic sulfur compounds. The two species, *C. limicola* and *C. thiosulfatophilum*, oxidize sulfides to elementary sulfur in the light under complete anaerobiosis at pH 7.5; the sulfur precipitates in the exocellular medium

$$CO_2 + 2H_2S \xrightarrow{\text{light}} (CH_2O) + 2S^0 + H_2O$$

Then, in a second step, they carry out oxidation to sulfate

$$3CO_2 + 2S^0 + 5H_2O \xrightarrow{\text{light}} 3(CH_2O) + 2H_2SO_4$$

The latter reaction does not proceed very far in *C. limicola* and therefore appreciable quantities of elementary sulfur accumulate in the cultures of this organism. The main difference between the two species, however, is that only *C. thiosulfatophilum* can assimilate carbon dioxide while using thiosulfate as electron donor:

$$2CO_2 + Na_2S_2O_3 + 3H_2O \xrightarrow{\text{light}} 2(CH_2O) + Na_2SO_4 + H_2SO_4$$

The two species have a hydrogenase and use molecular hydrogen as electron donor for photosynthesis:

$$2H_2 + CO_2 \xrightarrow{\text{light}} (CH_2O) + H_2O$$

The purple sulfur bacteria of the genus *Chromatium* have the same biochemical properties as *C. thiosulfatophilum* and assimilate carbon dioxide photosynthetically with the oxidation of sulfides, elementary sulfur, thiosulfate, or molecular hydrogen. They differ from the green bacteria in that their cells contain very obvious granules of elementary sulfur and in their ability to use as electron donors several organic compounds, among them mono- and dicarboxylic acids oxidized to CO_2. This mode of development in which photosynthesis is coupled with the anaerobic oxidation of an organic substrate is shared by the purple non-sulfur bacteria or Athiorhodaceae, photo-heterotrophic organisms whose metabolism was recently reviewed in detail by Elsden (*94*). Although otherwise very similar to the Athiorhodaceae, the purple sulfur bacteria cannot develop heterotrophically in the dark with their oxidizable organic substrates as source of energy.

4. *Heterotrophic Sulfo-oxidizing Bacteria*

Guittoneau (*95*) pointed out that many soil microorganisms oxidize sulfides, thiosulfate, and polythionates to sulfate. Starkey (*96*) isolated from garden earth several heterotrophic bacterial cultures which oxidized thiosulfate. According to Sijderius (*97*), these organisms are distributed very widely in nature and play a more significant role in inorganic sulfur metabolism than even the thiobacilli. This author divides the thiosulfate-oxidizing heterotrophs into two groups. One, which comprises the *Pseudomonas* species, oxidizes thiosulfate to tetrathionate with the aid of a constitutive enzyme system in a reaction which tends to make the medium more alkaline:

$$2Na_2S_2O_3 + H_2O + \tfrac{1}{2}O_2 \rightarrow Na_2S_4O_6 + 2NaOH$$

The other group, of which *Micrococcus denitrificans* is a typical example, uses adaptive enzymes to carry out oxidation to sulfate and free sulfuric acid, with pronounced acidification of the medium:

$$Na_2S_2O_3 + H_2O + 2O_2 \rightarrow Na_2SO_4 + H_2SO_4$$

B. Biochemistry of Sulfur Oxidation by Microorganisms

1. *Sulfide*

The elementary sulfur which accumulates in cells of Beggiatoaceae and Thiorhodaceae or extracellularly in cultures of Chlorobacteriaceae obviously is an intermediate in the oxidation of sulfide by these organisms. A transitory accumulation of sulfur is also found in the intracellular vacuoles of *T. thiooxidans* and in cultures of *T. thioparus* on sulfide-containing media. In thiobacilli, however, elementary sulfur may perhaps not be an intermediate in sulfide oxidation but may be produced in a secondary reaction. In fact, the formation of sulfur from sulfide is variable and Vishniac and Santer (*38*) found, moreover, that when cells of *T. thioparus* are incubated in the presence of radioactive sulfide, the label is found after a minute in polythionate and thiosulfate, without evidence of the formation of elementary sulfur.

2. *Sulfur*

T. thiooxidans grows rapidly on media containing a suspension of powdered sulfur as the sole energy source. That other species of thiobacilli, particularly *T. thioparus* and *T. denitrificans*, also utilize elementary sulfur is demonstrated by the fact that the often quite abundant precipitates of sulfur present in cultures during the early period of incubation have disappeared at the end of the growth phase and are thus oxidized secondarily. Several hypotheses have been advanced to explain how bacteria can metabolize an exogenous substrate as insoluble as elementary sulfur. Vogler and Umbreit (*98*) showed that the oxidation of S^0 by *T. thiooxidans* requires direct contact of the bacterial cells with sulfur. They postulated that sulfur enters the cells by being dissolved in a lipide globule attached to one end of the bacterium. However, electron micrographs of *T. thiooxidans* (*99*) failed to confirm the existence of such fat globules. According to another hypothesis, sulfur is solubilized by an exocellular reduction process or by contact with the bacterial wall. Starkey (*100*) does not consider an extracellular reduction of sulfur as likely but, in contrast, Vishniac and Santer (*38*) think it possible that sulfur is reduced by glutathione or another sulfhydryl compound and is then transported across the cell wall as free or combined sulfide.

Whatever its mechanism of entry into the cell, it is certain that sulfur is then oxidized successively to thiosulfate, polythionates, and sulfate. These intermediates were demonstrated by Guittoneau and Keilling (*101*) in soils experimentally enriched with elementary sulfur. According to Vishniac and Santer (*38*), thiosulfate is formed by the condensation

of sulfur and sulfite by a process analogous to that suggested by Fromageot (*102*) for the formation of thiosulfate in mammals.

3. *Thiosulfate*

Tetrathionate was shown by Vishniac (*103*) to be the first intermediate in the oxidation of thiosulfate on the basis of various indirect proofs such as the kinetic evolution of oxygen consumption during Warburg oxidation of thiosulfate by resting cells of *T. thioparus*. Further support is given by the finding that most thiobacilli oxidize tetrathionate, which may undergo transitory accumulation when these organisms oxidize thiosulfate under certain experimental conditions. More recently, Trudinger (*104*) demonstrated the formation of tetrathionate directly with extracts of resting cells of *Thiobacillus X*, an organism which closely resembles *T. thioparus*.

Ostrowski and Krawczyk (*105*) studied the mode of incorporation of radioactivity into elementary sulfur and into cell constituents produced during the growth of *T. thioparus* in the presence of thiosulfate labeled specifically on the "external" sulfur atom of the molecule:

$$(S^{35}—SO_3)^{2-}$$

On the basis of their results, they concluded that the "internal" sulfur atom of thiosulfate, i.e., the one carrying the sulfonic group, does not enter the cell and is removed from the molecule directly as sulfate ion which remains in the exocellular medium. According to Skarzynski and Ostrowski (*39*), the sulfur atom carrying the sulfhydryl group passes through the cell wall and combines with an unidentified acceptor to give rise to organic compounds which are postulated to be the precursors of cysteine, cystine, methionine, and thioctic acid. The final oxidation of the "external" sulfur atom of thiosulfate to sulfate would then take place after passage through these organic forms.

Trudinger (*104*) also employed thiosulfate molecules labeled with S^{35} in the "internal" or "external" position but did not confirm the conclusions of the preceding authors. In fact, he found no isotope exchange between the internal and external sulfur atoms in the tetrathionate, formed during these experiments; this shows that tetrathionate is produced in the cell by a process involving both sulfur atoms of thiosulfate,

$$2(\overset{1}{S}—\overset{2}{SO_3})^{2-} \rightarrow (O_3\overset{2}{S}—\overset{1}{S}—\overset{1}{S}—\overset{2}{SO_3})^{2-}$$

and consequently both must have effectively entered the cells.

By reducing to 10 seconds the incubation time of resting cells of *Thiobacillus X* with labeled thiosulfate, Trudinger (*104*) established the formation of another sulfur compound, in addition to tetrathionate,

which represents about 5% of the total incorporated radioactivity and has been identified as trithionate. This observation confirms the opinion of Vishniac and Santer (*38*), based on a series of indirect proofs, that oxidation of tetrathionate is followed by trithionate formation:

$$3S_4O_6^{2-} + 2.5\ O_2 + H_2O \rightarrow 4S_3O_6^{2-} + 2H^+$$

The later steps, between trithionate and sulfate, are still entirely unknown. Dithionate is not a likely intermediate since it is oxidized by *T. thioparus* only at a very slow rate (*106*) and is not degraded in soils first perfused with thiosulfate (*107*).

4. *Formation of Free Sulfur*

In cultures of *T. thioparus* on thiosulfate, especially if forced aeration is employed, a precipitate of free sulfur is often seen which is metabolized secondarily by the bacteria and disappears completely in the end. Since elementary sulfur is in an oxidation state inferior to that of thiosulfate, its formation under these conditions appears to be paradoxical. Tamiya *et al.* (*108*) showed that this finding can be explained by nonenzymatic reactions among polythionates. Tetrathionate, the first intermediate in thiosulfate oxidation, undergoes a spontaneous dismutation to trithionate and pentathionate, and the latter, which is quite unstable, decomposes to tetrathionate and elementary sulfur:

$$2S_4O_6^{2-} \rightarrow S_3O_6^{2-} + S_5O_6^{2-}$$
$$S_5O_6^{2-} \rightarrow S_4O_6^{2-} + S^0$$

Vishniac and Santer (*38*) consider these nonenzymatic reactions among polythionates as the effective mechanism for the formation of elementary sulfur in cultures of thiobacilli on thiosulfate. However, in contrast to Tamiya *et al.* (*108*), who explain the normal production of trithionate in this way, the former authors consider them as wasteful side reactions by which substrate is lost.

5. *Role of the Cytochromes*

Newton and Kamen (*109*) extracted and purified a cytochrome from cells of *Chromatium* sp. which had the spectroscopic properties of cytochrome c but was distinguished from it by being autoxidizable and having an oxidation-reduction potential ($E'_0 = -0.040$ volt) similar to that of the type-b cytochromes. Differential spectra obtained on *Chromatium* suspensions in the light and in darkness seem to indicate a functional role for this cytochrome in the transport of electrons between the photochemical reaction and the oxidation of sulfur compounds.

Cytochromes analogous to that of *Chromatium* have been demon-

strated in green sulfur bacteria, particularly in *Chlorobium limicola* by Kamen and Vernon (*110*) and in *C. thiosulfatophilum* by Gibson and Larsen (*111*).

Several cytochromes are also present in the thiobacilli. Klimer *et al.* (*112*) extracted single cytochrome s from *T. thioparus*. Despite its close physiological similarity to this organism, *Thiobacillus X* has a different cytochrome constitution, exhibiting several soluble cytochromes of type c, as shown by Trudinger (*113*). One of these (553.5 mμ) is autoxidizable, though its oxidation-reduction potential is strongly positive ($E'_0 = +0.210$ volt) and is reduced rapidly by thiosulfate at pH 7 in the presence of the thiosulfate-oxidizing enzyme extracted from the same bacteria. The subcellular particles which are sedimented at high speeds contain other insoluble cytochrome compounds and a cytochrome oxidase; in the presence of the soluble cytochrome 553.5 and the thiosulfate-oxidizing enzyme, the oxidation of thiosulfate can thus be coupled with the consumption of molecular oxygen.

Aubert *et al.* (*114*) extracted and purified a soluble cytochrome c from *T. denitrificans* which is not autoxidizable ($E'_0 = +0.270$ volt) and constitutes a remarkably large proportion (3.5%) of the dry weight of the bacteria. The same authors (*115*) showed that in whole cells this cytochrome takes part in the transport of electrons between the oxidation of thiosulfate and the reduction of nitrate to N_2. Moreover, they found that cell-free extracts of *T. denitrificans* exhibit slight thiosulfate oxidase activity but contain a very active sulfite reductase. This is the first bit of experimental evidence in support of an intermediary role of sulfite in thiosulfate oxidation by thiobacilli. Like the sulfite oxidase of dog liver, that of *T. denitrificans* requires hypoxanthine as a cofactor. It is coupled to oxygen by cytochrome c and a cytochrome oxidase of classical type, which is also present in extracts.

6. *Phosphorylations*

In discussing the mechanism of assimilation of CO_2 by chemo-lithotrophic bacteria in general, it was pointed out that the thiobacilli couple sulfur oxidation and CO_2 incorporation energetically by way of high-energy phosphate bonds. The formation of ATP from ADP and inorganic phosphate by cells of *T. denitrificans* was demonstrated experimentally by Milhaud *et al.* (*116*) with the use of P^{32}.

In sulfo-oxidizing bacteria, phosphorylations probably are involved not only in energy transport but also in the intermediary metabolism of the sulfur compounds themselves. The first observation along these lines was made by Knaysi (*117*), who noted the formation of intracellular vacuoles containing both sulfur and volutin (polymetaphosphates) dur-

ing the oxidation of sulfur by *T. thiooxidans*. These inclusions had previously been described as lipide globules since the sulfur gave them the same staining properties and affinity for iodine as are routinely used for the histochemical characterization of fats. Later, Barker and Kornberg (*118*) confirmed the accumulation of easily hydrolyzable polyphosphates by cells of *T. thiooxidans*. Vishniac and Santer (*38*) consider the role of these polymetaphosphates to be essentially that of energy storage. The concomitant content of phosphorus and sulfur of the vacuoles may also represent the transitory accumulation of certain intermediates in the oxidation of sulfur.

Santer and Vishniac (*119*) showed that in the absence of inorganic phosphate the oxidation of thiosulfate and tetrathionate by *T. thioparus* does not go to completion as shown by the fact that under these conditions the cells consume only 70% of the O_2 consumed in the presence of phosphate, which is also the stoichiometric amount for the complete oxidation to sulfate. Inorganic phosphate may be replaced by arsenate. According to Margulies and Santer (*120*), the oxidation of thiosulfate or tetrathionate in the absence of phosphate yields several other sulfur compounds in addition to sulfate which have been separated by paper chromatography but not identified. These findings suggest that inorganic phosphate is involved in the oxidation of certain intermediates which in its absence cannot be metabolized further and therefore accumulate. Vishniac and Santer (*38*) separated by chromatography the products formed by *T. thioparus* in the presence of both thiosulfate-S^{35} and phosphate-P^{32}. One or several doubly labeled compounds were thus obtained; these were considered to be mixed anhydrides of the type

$$R—S—O—PO_3^{2-}$$

which can transfer their phosphate bond secondarily to ADP to yield ATP. This hypothesis gained support from the demonstration of phosphorylated compounds of this type in other organisms. Robbins and Lipmann (*121*) and Wilson and Bandurski (*122*) showed that yeast and mammals activate sulfate by a process involving first the formation of adenosine-5′-sulfophosphate (APS) and then that of adenosine-3′-phospho-5′-sulfophosphate (PAPS):

(1) $$SO_4^{2-} + ATP \rightarrow APS + PP(\text{pyrophosphate})$$

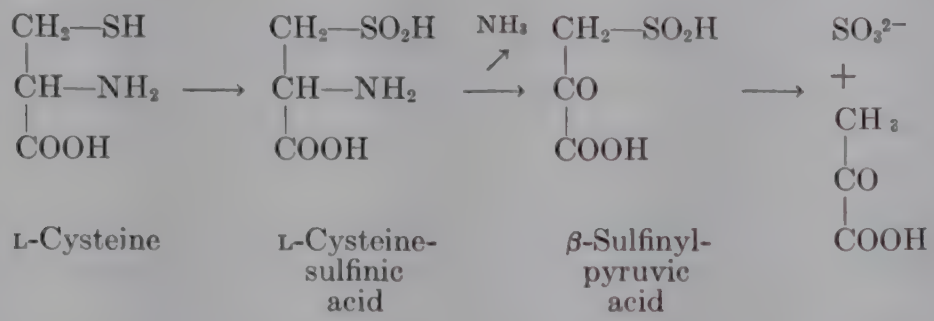
(2) $$APS + ATP \rightarrow PAPS + ADP$$

The sulfo-oxidizing bacteria may be expected to carry out all or part of this process. Quite recently, Peck (*123*) showed that the reduction of sulfate to sulfite by the sulfo-reducing bacterium *Desulfovibrio desulfuricans* involves the first of these two reactions, i.e., the formation of APS.

but not the second leading to the production of PAPS, the "active sulfate" of Robbins and Lipmann.

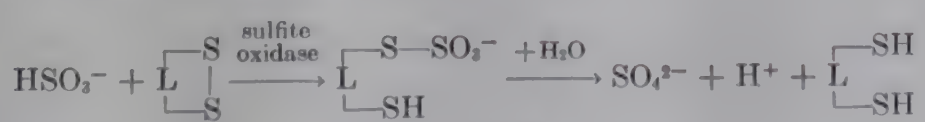
C. Oxidation of Sulfur and Sulfur Compounds in Mammals

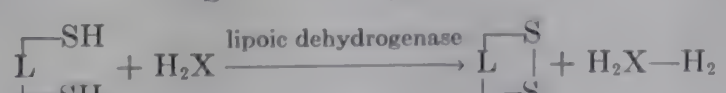
In mammals, one of the major routes of mineralization of organic sulfur leads to the formation of sulfite, which is derived in particular from cysteine by way of cysteinesulfinic acid and β-sulfinylpyruvic acid (*124*):



Sulfur thus mineralized is not eliminated in the urine as sulfite but rather as sulfate, free or conjugated in aromatic esters. Similarly, sulfur derived from the desulfhydration of cysteine or hydrogen sulfide injected into the veins of dogs (*125*) is also excreted in the urine as sulfate. Sulfide or elementary sulfur are most probably oxidized to sulfate through thiosulfate (*125*) which is constantly present in small amounts in the urine of normal animals, but the enzymatic steps of this process are not yet fully understood.

Fridovich and Handler (*126*) isolated a sulfite oxidase from dog liver which converts sulfite to sulfate by a complex process involving thioctic acid and hypoxanthine as cofactors. This enzyme is distinct from xanthine oxidase and is finally coupled with a flavin nucleotide terminal respiratory system by a complex chain of electron carriers. The general scheme of the process follows:





(3) $H_2X—H_2 + DPN^+ \rightarrow H_2X + DPNH + H^+$

(4) $DPNH + H^+ + FAD \rightarrow DPN^+ + FADH_2$

(5) $FADH_2 + O_2 \rightarrow FAD + H_2O_2$

Reaction 1, catalyzed by sulfite oxidase, consists of the formation of an addition compound between sulfite and oxidized thioctic or lipoic acid, which upon hydrolysis yields sulfate and reduced lipoic acid or dimer-

captodiquate. The cofactor is reoxidized by hypoxanthine (H.X) whic
is converted to an as yet unidentified form (Reaction 2). The late
stages involve the transfer of electrons to diphosphopyridine nucleotid
(Reaction 3), then to flavin adenine dinucleotide (Reaction 4), an
finally the reoxidation of reduced flavin by oxygen with the formatio
of hydrogen peroxide (Reaction 5). At present, it is not known if th
exergonic process gives rise to high-energy phosphate bonds which th
organism can use for other physiological functions. From the point
view of comparative biochemistry, it is of interest that an enzyme sy
tem similar to the one just described in mammalian liver has also be
demonstrated in the chemo-lithotrophic bacterium *T. denitrificans* (113

IV. Oxidation of Molecular Hydrogen

The ability to oxidize molecular hydrogen is tied to the action of
specific enzyme, hydrogenase, which activates the biatomic molecule
gaseous hydrogen and reversibly catalyzes the reaction:

$$H_2 \xrightarrow{\text{hydrogenase}} 2H^+ + 2e^-$$

This enzyme is almost exclusively present in bacteria, with the excepti
of a few other organisms (i.e., some algae). Among the bacteria, ho
ever, hydrogenase is very widely distributed; it is found not only
chemo-lithotrophic organisms capable of utilizing the aerobic or a
aerobic oxidation of H_2 as sole energy source but also in phototrop
and chemo-heterotrophic organisms where hydrogenase assumes vari
functions and takes part in various metabolic pathways.

The abundant literature on the different metabolic functions
hydrogenase and the properties of microorganisms endowed with it w
reviewed in great detail by Gest (*127*) some years ago.

A. Organisms Possessing Hydrogenase

1. *Chemo-lithotrophic Bacteria*

a. Hydrogen Bacteria. These are autotrophs which use as ener
source the aerobic oxidation of hydrogen gas:

$$H_2 + 0.5\,O_2 = H_2O \qquad (-\Delta F^\circ_{298} = 57.4 \text{ kc}$$

Since this reaction synthesizes water from detonating gas, the bacte
which can carry it out are often called *Knallgasbakterien* by Ger
authors.

The genus *Hydrogenomonas* (*81*) comprises the type species *H. g*
autrophus, the first form described by Kaserer (*128*) in 1908, and sev
other species including *H. vitrea* and *H. flava*, described by Nikle

20) in 1910. As these organisms are hard to cultivate and maintain artificial cultures their metabolism was little studied until the ore recent discovery, by Schatz and Bovell *(130)* in 1952, of a new ecies, *H. facilis*, which can be obtained easily in massive culture and s permitted rapid progress in the study of its biochemical properties.

Hydrogenomonas are nonsporulated eubacteria, short, Gram-negative alli possessing one or several polar flagellae. Their morphology is ite similar to that of the nitrifying bacteria and the thiobacilli, these ree groups are therefore classed together in the family *Nitrobacteriaceae (61)*. Physiologically, they are strict aerobes and facultative totrophs which can grow by using a certain number of organic compounds, particularly acetate, lactate, C_4-dicarboxylic acids, and glucose energy and carbon sources. In these organic media, they can grow in 0% O_2, but their autotrophic growth in an atmosphere containing both , and O_2 requires only a partial oxygen tension of about 3% since, as ll be seen below, hydrogenase is strongly inhibited by oxygen.

According to Nádenski *(129)*, *H. agilis* can grow anaerobically, in e absence of free oxygen, in nitrate-containing media. This organism, en, would behave exactly like the sulfo-oxidizing *Thiobacillus denitrifins*. *H. facilis* cannot utilize this "nitrate respiration" for anaerobic owth but nonetheless can utilize nitrate instead of ammonium salts nitrogen source and is able to reduce nitrate to nitrite in an H_2 mosphere in nonproliferating suspensions *(130)*.

The influence of culture conditions on the formation of hydrogenase cells of *Hydrogenomonas* was discussed above (Section I,D).

b. Sulfate-Reducing Bacteria. These organisms were discovered by ijerinck *(131)* in 1895 and now constitute the genus *Desulfovibrio J)*. They are strict anaerobes which employ sulfate as electron acptor and reduce it to hydrogen sulfide. The type species, *D. desulfuricans*, grows heterotrophically, using only a small number of organic mpounds, such as pyruvate and lactate, as energy and carbon sources. ith the latter substrate, which is most frequently employed for the ltivation of sulfate-reducing bacteria, the metabolic balance sheet is follows:

$$CH_3 \cdot CHOH \cdot COOH + 2H_2O \xrightarrow{\text{dehydrogenase}} 2CH_3 \cdot COOH + 2CO_2 + 8H^+ + 8e$$

$$8H^+ + 8e + H_2SO_4 \xrightarrow{\text{sulfate reductase}} H_2S + 4H_2O$$

$$CH_3 \cdot CHOH \cdot COOH + H_2SO_4 \rightarrow 2CH_3 \cdot COOH + 2CO_2 + H_2S + 2H_2O$$

Baars *(132)* described another species, *D. rubentschikii*, which carries the oxidation of organic substrates all the way to CO_2 and can util-

ize acetate and the lower fatty acids; however this species was not re-isolated and its existence still requires proof.

D. desulfuricans was one of the first organisms in which Stephenson and Stickland (*133*) demonstrated the presence of a hydrogenase which enables the bacterium to reduce sulfate by utilizing gaseous hydrogen:

$$4H_2 + H_2SO_4 \rightarrow H_2S + 4H_2O$$

The free energy produced by this reaction, taking into account the hydrolysis of the resultant sulfide (*134*), is 29.7 kcal. per mole H_2 consumed. Starkey and Wight (*135*), Butlin and Adams (*136*), and Senez and Volcani (*137*) reported experimental results showing that *D. desulfuricans* can utilize this energy source to grow on a completely inorganic medium and must therefore be classed as a facultative chemolithotroph; but the assimilation of CO_2 by this organism and its incorporation into the cell constituents has not yet been demonstrated.

D. desulfuricans is remarkable because it is the only strictly anaerobic bacterium with a cytochrome, demonstrated by Postgate (*134*) and described as cytochrome c_3. It shares the spectroscopic properties of mammalian cytochromes c but differs by its very low oxidation-reduction potential ($E'_0 = -0.205$ volt) and its autoxidizability. Like mammalian cytochrome c, cytochrome c_3 has a molecular weight around 12,000 but its molecule is bifunctional and contains two hemin groups. Postgate (*134*) adduced evidence for the functional participation of cytochrome c_3 in electron transport from hydrogenase to sulfate and other inorganic sulfur compounds.

2. *Phototrophic Organisms*

As seen above (cf. Sections I,B,2 and III,A,3), the green and purple sulfur bacteria possess hydrogenase and oxidize H_2 under anaerobiosis in the light, but do not utilize the resultant energy for their biosynthetic activities.

The same mode of photosynthesis is carried out by the nonsulfur purple bacteria or Athiorhodaceae. These photo-heterotrophs also contain a hydrogenase, demonstrated in several members of the group and extracted and purified by Gest (*138*) from cells of *Rhodospirillum rubrum*. Under certain experimental conditions, the Athiorhodaceae, like the purple sulfur bacteria, produce gaseous hydrogen during photosynthesis. This photoevolution of H_2, discovered by Gest and Kamen (*139*), occurs when cells are incubated anaerobically in the presence of various organic substrates such as acetate, propionate, and *n*-butyrate. It is certainly connected with the activity of the hydrogenase which mediates the liberation of gaseous hydrogen, but its mechanism has

not yet been clarified completely; in particular, it remains to be seen whether the liberated hydrogen comes from the photochemical reaction, from the oxidation of organic substrates, or from both of these sources at the same time (*94*).

Among the Athiorhodaceae, hydrogenase is involved in yet another metabolic process. In the dark, resting cells of *R. rubrum* (*138*) consume H_2 and simultaneously reduce a variety of acceptors, e.g., oxygen, nitrate, thiosulfate, ferricyanide, fumarate, and dyes; in this organism, then, the function of the hydrogenase is not necessarily connected with photosynthesis. However, these cells cannot use this mode of oxidation of hydrogen as an energy source to reduce CO_2 and develop chemolithotrophically.

In addition to chlorophyll-containing bacteria, a hydrogenase has been demonstrated in other photosynthetic organisms, particularly in algae. Gaffron (*140*) showed that the green alga *Scenedesmus* is capable of photoevolution of H_2 and acquires strong hydrogenase activity when incubated in the dark under hydrogen. The adapted cells consume H_2 either in the light, where they carry out photosynthesis similar to that of bacteria, or in the dark, where they can use the energy derived from the anaerobic oxidation of H_2 to reduce CO_2 chemo-lithotrophically. Photoevolution of H_2 has also been reported in another green alga, *Chlamydomonas moewusii,* by Frenkel (*141*), and in various blue-green brown, and red algae by Frenkel and Rieger (*142*). In these algae, however, consumption of molecular hydrogen has been observed neither in the dark nor in the light.

The physiological relationships between hydrogenase and photosynthesis are complicated by the fact that in certain phototrophic organisms the consumption or evolution of H_2, i.e., the reactions in which hydrogenase participates, are dissociated or take place only in the light, whereas in others all the reactions can occur either in light or darkness. According to Gest (*143*), who discusses this problem in detail, dependence on light may be secondary and connected with the photochemical formation of an organic precursor by which the electrons are transferred to the hydrogenase system.

Krasna and Rittenberg (*144*) have stated that in biochemical evolution hydrogenase may have preceded the appearance of the photosynthetic system of higher plants, i.e., the system leading to the formation of free oxygen. Moreover, the same authors pointed out that hydrogenase may be a ferroprotein and that its molecule may be the precursor of the hematinic respiratory enzymes of animals such as the cytochromes, catalase, and peroxidase. In the framework of this phylogenetic theory, the chlorophyll-containing bacteria and the hydrogenase-containing algae

are evolutionary remnants of particular interest to comparative biochemistry.

3. *Heterotrophic Bacteria*

Numerous chemo-heterotrophic bacteria which are strict or facultative anaerobes possess a hydrogenase and can oxidize H_2 or, on the contrary, liberate molecular hydrogen from various organic substrates (Table IX). Some of these reactions are strongly exothermic, but none of them are used by these heterotrophic microorganisms as an energy source for growth and hence it will suffice here to mention only those most important for comparative biochemistry; a more complete description is given in the significant general review by Gest (*127*).

Among the reactions listed in Table IX, the so-called "phosphoroclastic" decarboxylation of pyruvate is one of the most interesting. It is carried out by most of the saccharolytic species of *Clostridia*, or strictly anaerobic sporulated bacteria, and in particular by *Clostridium butylicum* (*145*) and by the nitrogen-fixing species *C. pasteurianum* (*146*). The reversibility of this process was demonstrated by the isotopic incorporation of CO_2 into the carboxyl group of pyruvate (*147*) and by the inhibitory effect of high partial pressures of H_2 on the decarboxylation of this keto acid. At first, the participation of hydrogenase was questioned because the cells of *C. butylicum* cannot reduce methylene blue in an H_2 atmosphere and thus appear to lack this enzyme. Later, however, a very active hydrogenase was demonstrated in *C. acetobutylicum*, which carries out this reaction, and even in *C. butylicum* itself by Peck and Gest (*148*) with a new assay method based on the liberation of H_2 from reduced methyl viologen. The phosphoroclastic reaction has also been found in other bacteria possessing an active hydrogenase, especially in *Micrococcus lactilyticus* (*Veillonella gazogenes*) (*127*) and *D. desulfuricans* (*149, 150*).

The facultative anaerobes of the colon-aerogenes group decompose formate to CO_2 and H_2 by a process discovered in *Escherichia coli* by Stephenson and Stickland (*151*) and then shown to be reversible by Woods (*152*). The former authors called this enzyme system formic hydrogenlyase and proposed a two-stage mechanism for its action—the first step involving the dehydrogenation of formate by a formic dehydrogenase, and the second the liberation of hydrogen atoms as H_2 by a hydrogenase:

$$\text{(1)} \qquad HCOOH \xrightleftharpoons{\text{formic dehydrogenase}} CO_2 + 2H^+ + 2e$$

$$\text{(2)} \qquad 2H^+ + 2e \xrightleftharpoons{\text{hydrogenase}} H_2$$

TABLE IX

METABOLIC REACTIONS OF ORGANIC SUBSTANCES IN WHICH HYDROGENASE IS INVOLVED

Reactions		Organisms	References[a]
Methane formation	$CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O$	Methane bacteria (*Methanobacterium omelianskii, M. formicicium, Methanosarcina barkerii, Methanococcus vannielii*);	(*a*)
	$CO + 3H_2 \rightarrow CH_4 + H_2O$	*M. barkerii*	(*b*)
Formic-hydrogenlyase system	$CO_2 + H_2 \rightleftharpoons HCOOH$	Colon-aerogenes bacteria (*Escherichia coli, Aerobacter aerogenes, A. cloacae*)	(*c, d, e*)
Phosphoroclastic reaction	Pyruvate + $P_i \rightleftharpoons$ Acetyl phosphate + $CO_2 + H_2$	*Clostridium butylicum, C. acetobutylicum* and other clostridia;	(*f, g*)
		Desulfovibrio desulfuricans;	(*h*)
		Micrococcus lactilyticus	(*i*)
Reduction of aldehydes and acids	H_2 + Acetaldehyde $\rightarrow$ Ethanol	*Clostridium kluyveri*	(*j*)
	$4H_2 + 2CO_2 \rightarrow CH_3 \cdot COOH + 2H_2O$	*Clostridium aceticum*	(*k*)
Reductive deamination of amino acids	$H_2 + R \cdot CH(NH_2) \cdot COOH \rightarrow NH_3 + R \cdot CH_2 \cdot COOH$	*Clostridium sporogenes*	(*l*)
Reduction of purines	H_2 + Xanthine $\rightleftharpoons$ Hypoxanthine	*Micrococcus lactilyticus*	(*m*)
Reduction of fumarate	H_2 + Fumarate $\rightleftharpoons$ Succinate	*Proteus vulgaris*	(*n*)
		E. coli	(*o*)

[a] REFERENCES: (*a*) H. A. Barker, *Proc. Natl. Acad. Sci. U. S.* **29,** 184 (1943); (*b*) A. J. Kluyver and C. G. T. P. Schnellen, *Arch. Biochem.* **14,** 57 (1947); (*c*) M. Stephenson and L. H. Stickland, *Biochem. J.* **26,** 712 (1931); (*d*) M. Stephenson, *Ergeb. Enzymforsch.* **6,** 139 (1937); (*e*) E. J. Ordal and H. O. Halvorson, *J. Bacteriol.* **38,** 199 (1939); (*f*) H. J. Koepsell and M. J. Johnson, *J. Biol. Chem.* **145,** 379 (1942); (*g*) J. Wilson, L. O. Krampitz, and C. H. Werkman, *Biochem. J.* **42,** 598 (1948); (*h*) J. C. Senez, *Bull. soc. chim. biol.* **36,** 541 (1954); (*i*) A. T. Johns, *J. Gen. Microbiol.* **5,** 326 (1951); (*j*) E. R. Stadtman and H. A. Barker, *J. Biol. Chem.* **180,** 1117 (1949); (*k*) M. F. Utter and H. G. Wood, *Advances in Enzymol.* **12,** 41 (1951); (*l*) J. G. Hoogerheide and W. Kocholaty, *Biochem. J.* **32,** 949 (1938); (*m*) H. R. Whiteley and H. C. Douglas, *J. Bacteriol.* **61,** 605 (1951); (*n*) L. Farkas and E. Fischer, *J. Biol. Chem.* **167,** 787 (1947); (*o*) J. Lascelles and J. L. Still, *Proc. Linnean Soc. N. S. W.* **72,** 49 (1947).

This concept has been criticized because some strains of *E. coli* o *Aerobacter aerogenes* have hydrogenlyase activity but appear to lac formic dehydrogenase or hydrogenase. It was shown later by Ordal an Halvorson (*153*) that the absence of these enzymatic activities is due no to the absence of the enzymes themselves but rather to the lack o transport systems required to permit demonstration of hydrogenase o dehydrogenase activities by standard techniques. Peck and Gest (*154* made a detailed study of the enzyme constitution of the formic hydro genlyase system of *E. coli* and an atypical strain of *Klebsiella*. The concluded that the complete system involves four distinct component formic dehydrogenase, hydrogenase, and two transport agents calle X_1 and X_2.

Hydrogenase has also been demonstrated in other chemo-hetero trophic bacteria which neither consume nor liberate H_2 in their norm metabolism, particularly in the nitrogen-fixing strict aerobe *Azotobact vinelandii* (*155*), in *Acetobacter peroxydans* (*156*), *Bacillus delbruck* (*157*), and *Streptococcus faecalis* (*158*).

This brief analysis of the reactions carried out by heterotrophic ba teria with the consumption or production of H_2 allows us to isolate th major function of hydrogenase in most of these organisms. It appea that the physiological role of the enzyme is not the activation of th molecule of H_2 for oxidation to yield an energy source; on the contra it seems to function in the inverse sense, enabling the bacterium to eli inate in molecular form the hydrogen derived from the oxidation organic substrates. By this mechanism, hydrogenase gives to strict facultative anaerobes a means of oxidizing their substrates anaerobica in the absence of any exogenous hydrogen acceptor. As Gest (*127*) h pointed out, this physiological role is comparable to that of the respi tory chain, i.e., cytochromes–cytochrome oxidase, in aerobic organism

B. Properties and Constitution of Hydrogenase

1. *Methods of Investigation*

Stephenson and Stickland (*159*) were the first to demonstrate t existence, in suspensions of *E. coli* and other bacteria, of the enzy which catalyzes activation of H_2 specifically and which they cal hydrogenase. They found that in an H_2 atmosphere this enzyme duces a variety of acceptors, particularly methylene blue (MB) wh is converted stoichiometrically to a colorless leuco-derivative (MBH_2

$$H_2 + MB \xrightarrow{\text{hydrogenase}} MBH_2$$

This reaction permits the quantitative estimation of hydrogenase activity from the rate of decoloration of methylene blue by bacterial suspensions or extracts in Thunberg tubes under H_2. The reaction can also be followed colorimetrically or volumetrically by measuring H_2 consumption by the Warburg technique. Other acceptors may be employed, depending on the organism or enzyme preparation under study, e.g., ferricyanide, oxygen, nitrate, sulfate; other colored indicators such as safranine O, dichlorophenolindophenol, indigo disulfonate, or triphenyltetrazolium may be used.

The reaction catalyzed by hydrogenase

$$H_2 \rightleftharpoons 2H^+ + 2e$$

is that of the hydrogen electrode and has a very low oxidation-reduction potential ($E'_0 = -0.420$ volt at pH 7; $\Delta E'_0/\Delta pH = -0.060$ volt). Artificial acceptors are therefore useful whose potential lies closer to that of hydrogenase than that of methylene blue ($E'_0 = +0.011$ volt). For this reason, several authors have recommended the viologens, whose chemical and physicochemical properties were studied by Michaelis and Hill (*160*). In contrast to other oxidation-reduction indicators, the viologens are colorless in the oxidized state and colored in the reduced state; they fix only a single electron per molecule and have an unchanging potential with pH. The most commonly used H_2 acceptor for hydrogenase is benzyl viologen ($E'_0 = -0.359$ volt).

In contrast with dye-reduction procedures, Peck and Gest (*161*) described a new technique for the determination of hydrogenase activity based on the liberation of H_2 from a single-electron dye, methyl viologen ($E'_0 = -0.446$ volt) reduced by an excess of hydrosulfite. At pH 7.0, this indicator has an E'_0 below that of the hydrogenase-catalyzed reaction and consequently tends to be reoxidized by the enzyme with the removal of H_2. By this method it was shown that the cells of *C. butylicum* have high hydrogenase activity although they appear inactive by the conventional methylene blue reduction method. Similarly, the technique based on H_2 evolution gives higher values for hydrogenase activity than methylene blue assays for suspensions of *E. coli*, *C. pasteurianum*, and *M. lactilyticus*. However, *A. vinelandii* and *R. rubrum*, which reduce methylene blue in an H_2 atmosphere, do not release hydrogen from reduced methyl viologen.

It has been stressed that the number and nature of dyes reduced vary in an apparently erratic manner from one organism to the next. One of the reasons for this is the absence of a cofactor or intermediary carrier required for the reduction of a given acceptor. Curtis and Ordal (*162*) showed, moreover, that methylene blue and other dyes reversibly

inhibit certain hydrogenase preparations. According to Gest (*138*), methylene blue inhibits only the soluble hydrogenases and has no effect on particulate hydrogenases. Further, the inhibitory activity of the dye was attributed to metallic impurities. It should be noted, however, that the oxidation-reduction potential of methylene blue is relatively high and that, in systems where the dye is introduced in the oxidized state, certain hydrogenases endowed with particular sensitivity to oxidation may be inactivated by the simple influence of the dye on the oxido-reduction potential of the medium.

In 1934, Farkas *et al.* (*163*) showed that hydrogenase catalyzes the exchange reaction between deuterium and water:

$$D_2 + H_2O \rightleftharpoons HD + HDO$$

This reaction is studied by measuring with a mass-spectrograph the enrichment of water in deuterium in systems where an aqueous suspension of hydrogenase is placed under a D_2 atmosphere; this was employed

TABLE X

Comparison of Exchange and Reducing Activities of Different Organisms

Organism	$-Q_{H_2}$ [b]		Ratio		
	Exchange reaction	Methylene blue reduction	Methylene blue/ exchange	Benzyl viologen/ exchange	Reduced benzyl viologen/ exchange
E. coli	41,000	24,000	0.60	0.069	0.011
P. vulgaris	6,000	4,500	0.75	0.039	0.064
C. pasteurianum	2,500	9,200	3.6	1.57	0
D. desulfuricans	40,000	58,000	1.45	0.45	0.077
Acetobacter peroxydans	170	670	3.9	—	—
R. rubrum	24	153	6.4	0.28	0
Azobacter vinelandii	260	7,500	28.5	—	—

[a] From A. I. Krasna and D. Rittenberg, *Proc. Natl. Acad. Sci. U. S.* **42**, 180 (1956).

[b] *Note:* Activities ($-Q_{H_2}$) of bacterial cells expressed in microliters of H_2 evolved, activated, or used per milligram of nitrogen and per hour.

extensively by Hoberman and Rittenberg (*164*). Further, Krasna and Rittenberg (*165*) showed that the exchange reaction is carried out by hydrogenase in parallel with the conversion of *para-* to *ortho*-hydrogen by the same enzyme. These two methods of hydrogenase assay require costly and complex instrumentation but have the theoretical advantage that the enzyme activity is measured in the absence of any exogenous acceptor.

Krasna and Rittenberg (*166*) compared that various assay methods

for several bacteria. They obtained the values shown in Table X, to which their own data on the rate of conversion of *para-* to *ortho*-hydrogen should be added. The latter, expressed as in the other assays in terms of $-Q_{H_2}$,* is 10,000 for *Proteus vulgaris* and only 25 for *R. rubrum.*

These observations and the entirely concordant findings of Peck *et al.* (*167*) show that hydrogenase activity varies considerably from one bacterial species to the next. In *D. desulfuricans,* the most active organism, the activity is indeed about two thousand times greater than in *R. rubrum.* Another finding is that none of the methods available at present invariably yield the highest or most accurate results. After all, it appears that despite the hopes placed on the exchange and conversion reactions none of the available methods really measures the true activity of the enzymes, i.e., the activation of the hydrogen molecule, under conditions which are independent of any accessory factor or system.

2. *Purification of the Enzyme*

The first cell-free extract with hydrogenase activity was obtained by Bovarnik (*168*) from *E. coli.* Later, crude active extracts were prepared from cells of other microorganisms, notably *R. rubrum* (*138*), *A. vinelandii* (*169*), *H. facilis* (*170*), and *Micrococcus aerogenes* (*162*). The attempts to purify these hydrogenases have been stymied by the fact that the enzyme was in particulate form (*171*).

Shug *et al.* (*172*) extracted a soluble hydrogenase from the aerobic nitrogen-fixing bacterium *Clostridium pasteurianum* and achieved considerable purification by standard protein separation techniques. Peck and Gest (*148*) extracted another soluble hydrogenase from *C. butylicum* and obtained purified preparations whose specific activity, measured by H_2 evolution from reduced methyl viologen, reached $5–9 \times 10^6$ μl. hydrogen liberated per hour per milligram nitrogen. Sadana and Jagannathan (*173*) purified the hydrogenase of *D. desulfuricans* and obtained a specific activity of 2.6×10^6, which was determined by methylene blue reduction.

According to Kondo *et al.* (*174*), the hydrogenase contained in crude extracts of *D. desulfuricans* is a particulate enzyme which sediments to the extent of 90% in about 2 hours of centrifugation at 100,000 *g.* These investigators solubilized this hydrogenase and those of *E. coli* and *P. vulgaris* by treatment with sodium deoxycholate, partial trypsin digestion, and ammonium sulfate precipitation.

The hydrogenase of *C. pasteurianum* (*172*) and the other purified

* $-Q_{H_2}$ equals microliters of H_2 consumed, evolved, exchanged, or converted per hour and per milligram of bacterial nitrogen.

preparations are relatively stable and can be maintained for seve
weeks at +2°, as long as they are kept without air, under hydroge
Fischer *et al.* (*175*) showed that hydrogenase is inhibited reversibly
oxygen, regaining full activity when O_2 is eliminated from the mediu
e.g., by hydrosulfite. On the basis of their observations, they postulate
that hydrogenase, like hemoglobin, might pass reversibly from its norm
form to an oxygenated, inactive form. Irreversible loss of activity, how
ever, results from prolonged exposure to air or addition of oxidants.

3. *Nature of the Prosthetic Group*

Following the work of Shug *et al.* (*172*), hydrogenase has be
considered a molybdoflavoprotein intimately related in structure to t
other metalloflavoproteins and to xanthine oxidase in particular. Th
concept is based on various experimental results, notably on the simu
taneous presence of molybdenum and flavins in every preparation,
gardless of the degree of purification of the hydrogenase. Another i
portant fact is that the hydrogenase of *C. pasteurianum*, when treat
with ammonium sulfate and dialyzed for a long time, loses a lar
part of its activity; this is restored by the addition of both FAD a
molybdenum as molybdic trioxide. The addition of the flavin and t
metal restores the cytochrome *c* reductase activity (i.e., the reduct
of this cytochrome in an H_2 atmosphere), but not the ability to redu
methylene blue, this indicates that the reduction of the dye requires
supplementary factor or electron carrier which is lost during purificati

Other authors have reported data supporting the idea that the me
involved in hydrogenase activity is ferrous iron. Whiteley and Or
(*176*) showed that the hydrogenase of *Micrococcus lactilyticus* wh
they separated from a xanthine oxidase present in the crude extracts w
inhibited by orthophenanthroline and reactivated by catalytic quanti
of Fe^{2+}. Hoberman and Rittenberg (*164*) concluded that the hydrog
ase they were studying was a ferroprotein complex and Waring
Werkman (*177*) noted that cells of *Aerobacter aerogenes* grown on ir
free media did not exhibit hydrogenase activity. Peck and Gest (*1
showed that Fe^{2+} activates the hydrogenases of *M. lactilyticus* and
butulinum and concluded that both iron and molybdenum were pres
in these enzymes.

Winfield (*178*) emphasized that an iron requirement or an act
tion by iron does not necessarily mean that this metal is directly
volved in the constitution of the hydrogenase and plays a functional
in the activation of H_2. Iron may have only a secondary role of ch
tion, similar to that observed in other metalloflavoproteins by Ma
et al. (*179*). Another possibility is that iron is not involved in hy

nase activity proper but rather in electron transport between this zyme and certain acceptors. This hypothesis would explain why the ture of the metal in question may vary from one author to the next, pending on the source of the hydrogenase and the nature of the sys- m under investigation.

Mechanism of Action

To represent the mechanism of action of hydrogenase, Green and ckland (*180*) proposed a scheme in which the biatomic hydrogen olecule would be dissociate to give rise first to monoatomic hydrogen H·) and then to protons (H^+) and electrons (e):

$$(1) \qquad H_2 \rightleftharpoons 2H\cdot$$
$$(2) \qquad 2H\cdot \rightleftharpoons 2H^+ + 2e$$

rama and Rittenberg (*165*) found that cells of *P. vulgaris* convert ra- to *ortho*-hydrogen if they are in suspension in H_2O, but not in avy water (D_2O). This fact does not fit with the scheme of Green d Stickland since their hypothesis requires that the molecular recom- nation of *para*-hydrogen must yield *ortho*-hydrogen or "normal" hy- gen in every instance and whatever solvent may be present in the cterial suspension. For this reason, Krasna and Rittenberg (*165*) pro- sed this scheme for the mechanism of hydrogenase:

$$H_2 + E \rightleftharpoons H{:}E^- + H^+$$

which E represents the enzyme and $H{:}E^-$ a complex between the zyme and an hydrogen atom. This scheme fits the experimental re- lts well and explains in particular that when the bacterial cells are spended in D_2O, where the concentration of H^+ is extremely low in mparison to the concentration of D^+ ions, molecular recombination nds to produce HD rather than H_2, which has no possible effect on e spin state of hydrogen. These authors postulated that the $H{:}E^-$ mplex or enzyme "hydride" is the actual reducing agent formed by drogenase.

Winfield (*178*) also adopted a scheme involving the formation of a mplex of hydrogenase with a single atom of the H_2 molecule, but stulated that two successive stages were likely to occur:

$$(1) \qquad H_2 + E \rightleftharpoons H{-}E{-}H$$
$$(2) \qquad H{-}E{-}H \rightleftharpoons H{:}E^- + H^+$$

Peck *et al.* (*167*) found that certain hydrogenases reduced dyes of e viologen group rapidly but did not carry out the reverse reaction, .. the liberation of H_2 from reduced methyl viologen. They also noted at *A. vinelandii* and *R. rubrum* carried out the exchange reaction much

less rapidly than the reduction of methylene blue. Based on these findings and on the activation of the hydrogenases of *M. lactilyticus* and *C. butylicum* by Fe^{2+}, Peck and associates describe hydrogenase as a ferromolybdoflavoprotein with two distinct prosthetic groups whose activities can be dissociated. One of these groups would carry out specifically the transfer of hydrogen to natural or artificial acceptors with two electrons while the other, which would involve both FAD and molybdenum, would transfer hydrogen specifically to acceptors with one electron, e.g., cytochrome c or the viologens. This second group was likewise implicated in the hydrogen-deuterium exchange reaction and the liberation of gaseous hydrogen.

The hypothesis which states that flavometalloenzymes may be multifunctional and that molybdenum may be involved only in hydrogen transport to acceptors with a single electron was also advanced by Shug *et al.* (*172*) for the hydrogenase of *C. pasteurianum* and by Mackler *et al.* (*181*) for xanthine oxidase.

5. *Natural Hydrogen Carriers Coupled Functionally with Hydrogenase*

The number and nature of the natural acceptors which are coupled with hydrogenase vary with the organism. Korkes (*182*) demonstrated a system coupling hydrogenase with the reduction of pyridine nucleotides in *C. kluyveri*. Analogous systems were observed by Peck and Gest (*183*) in *C. pasteurianum*, by Packer and Vishniac (*184*) in *H. ruhlandii* and by Wittenberger and Repaske (*185*) in *H. eutropha*. However, functional coupling between hydrogenase and pyridine nucleotides was not encountered in cells or extracts of *E. coli* (*138, 186*) or *D. desulfuricans* (*187*).

Korkes (*182*) showed that coupling with pyridine nucleotides requires a thermostable cofactor; this was recently studied exhaustively by Kinsky (*188*). His investigation established that the complete coupling system in *C. kluyveri* comprises a DPN reductase and the Korkes cofactor which most probably is a flavin but does not appear to be flavin mononucleotide (FMN) or flavin adenine dinucleotide (FAD). This cofactor can be replaced by artificial carriers such as benzyl viologen or safranine O or, in part, by FAD. The hydrogenase of *C. kluyveri* reduces FAD and the dyes directly, without the participation of any intermediary carrier, while in *H. ruhlandii* (*184*) catalytic amounts of DPN must be added to the extracts to permit the reduction of methylene blue or benzyl viologen to proceed. Since FAD is reduced rapidly by several hydrogenases, it is likely that in certain organisms the oxidation of H_2 is coupled to oxygen consumption by a flavin respiratory chain, but experimental support for this hypothesis is still lacking.

Little is known about the coupling of hydrogenase to the cytochromes. *Hydrogenomonas* probably has a cytochrome respiratory system and this is coupled functionally to the hydrogenase and the aerobic oxidation of H_2, but despite its intrinsic interest this problem has not yet been investigated. However, cytochromes have been shown to be present in several bacteria which have a hydrogenase, in particular in the phototrophic organisms *Chromatium* (*109*), *Chlorobium limicola* (*110*), *C. thiosulfatophilum* (*111*), *Rhodospirillum rubrum* (*189*), and in the aerobic nitrogen-fixing bacterium *Azotobacter vinelandii;* from the latter, Tissieres (*190*) extracted and purified two nonautoxidizable cytochromes c (c_4 and c_5) whose E'_0 values, respectively, are +0.300 and +0.320 volt. The functional role of these cytochromes as hydrogen carriers coupled to hydrogenase has not yet been studied.

Postgate (*134*) showed that the cytochrome c_3 of *D. desulfuricans* is very rapidly reduced by the hydrogenase of this organism and carries the hydrogen to systems which reduce sulfates and other sulfur compounds. Senez and Pichinoty (*187*) found that the cytochrome c_3 reduced by hydrogenase is reoxidized chemically, without the aid of specific reductases, by nitrite and hydroxylamine which are reduced to ammonia. This shows that the cytochromes can function as electron carriers in anaerobic systems, without any contact with free oxygen. It also indicates that, in the presence of a natural or artificial carrier with a suitable potential, hydrogenase can reduce certain substrates, such as inorganic nitrogen compounds, by nonspecific processes of chemical type.

6. *Interrelations between Hydrogenase and Nitrogen Fixation*

Lee and Wilson (*191*) noted that the cells of *A. vinelandii* were much richer in hydrogenase if they were derived from cells which had fixed atmospheric nitrogen than if they grew on an ammonium salt as nitrogen source. They also observed that gaseous hydrogen strongly inhibits the fixation of N_2 by these same bacteria. It was later found that H_2 inhibits nitrogen fixation by red-clover nodules (*172*) and the blue-green alga *Nostoc* (*192*), but definite evidence has not yet been obtained for the presence of a hydrogenase in the whole nodule of this legume, or in pure cultures of the saprophytic bacterium *Rhizobium trifolii* (*193*), or in the cells of *Nostoc* and of other Cyanophyceae (*142*).

These data led Wilson and his group (*194*) to postulate a direct relationship between nitrogen fixation and hydrogenase and to state that all hydrogenase-containing organisms should be able to fix nitrogen. The latter hypothesis was verified by Gest *et al.* (*195*) for the photolithotrophic bacterium *Rhodospirillum rubrum*, which had been known

to possess a hydrogenase and which these authors showed to fix N_2. The hydrogenase activity of *R. rubrum*, like that of *A. vinelandii*, is much larger if the cells have developed by fixation of gaseous nitrogen. Moreover, in this organism the interaction between the hydrogenase and the nitrogen-fixing system is further emphasized by the fact that the photoevolution of H_2 by resting cells from pyruvate and C_4-dicarboxylic acids is completely inhibited by N_2, showing that gaseous nitrogen can combine with the enzymatic system which carries out the photoevolution and which comprises the hydrogenase as one of its constituents.

Recently, Le Gall *et al.* (*196*) demonstrated that *D. desulfuricans*, a strict anaerobe with a very high hydrogenase activity, can fix nitrogen. It continues to do so at relatively high partial pressures of hydrogen but cannot grow chemo-lithotrophically in an $H_2 + N_2$ atmosphere on a medium devoid of combined nitrogen.

Shug *et al.* (*172*) performed an experiment that seemed to demonstrate directly the interaction between the hydrogenase of *C. pasteurianum* and the "nitrogenase," i.e., the nitrogen-fixing system, of the same organism. They took the difference spectrum of purified hydrogenase in pure H_2 and in the presence of a mixture of H_2 and N_2. Under these conditions, they obtained spectra with two absorption peaks which were interpreted as those of the first intermediate compound of nitrogen during its fixation. Analogous findings were made by Hamilton *et al.* (*197*) with extracts of *Azotobacter*.

Shug and associates assign to hydrogenase a role in nitrogen fixation similar to that predicted by Winfield (*178*) on theoretical grounds. "Nitrogenase" is considered a metalloflavoprotein whose active site involves two atoms of iron or molybdenum suitably spaced, on which the N_2 molecule would be fixed by simple or double covalent bonds. Hydrogenase, itself a metalloflavoprotein, would transfer hydrogen atoms to the two atoms of nitrogen thus fixed and activated, as shown in Fig. 3; these successive hydrogenations would finally lead to ammonia. In this theory, the inhibition of nitrogen fixation by hydrogen is explained by competition between nitrogen and hydrogen for the active sites of the nitrogenase.

Gest *et al.* (*195*) feel that in *R. rubrum* nitrogen fixation is also a reduction process and that in this photosynthetic organism the principal source of hydrogen or electrons is the photolysis of water. Active hydrogen derived from the photochemical reaction, in addition to that formed by hydrogenase when this enzyme disposes of suitable donors, would accumulate in a sort of pool of reducing power with a very low oxidation-reduction potential. The electrons would be directed from this pool either toward reduction and assimilation of N_2 or toward other

enzymatic processes of intermediary metabolism. The hydrogenase would assume the role of a safety valve in this system, permitting the elimination of excess hydrogen atoms in their molecular form. Concerning the inhibition of photoevolution of hydrogen by N_2, this phe-

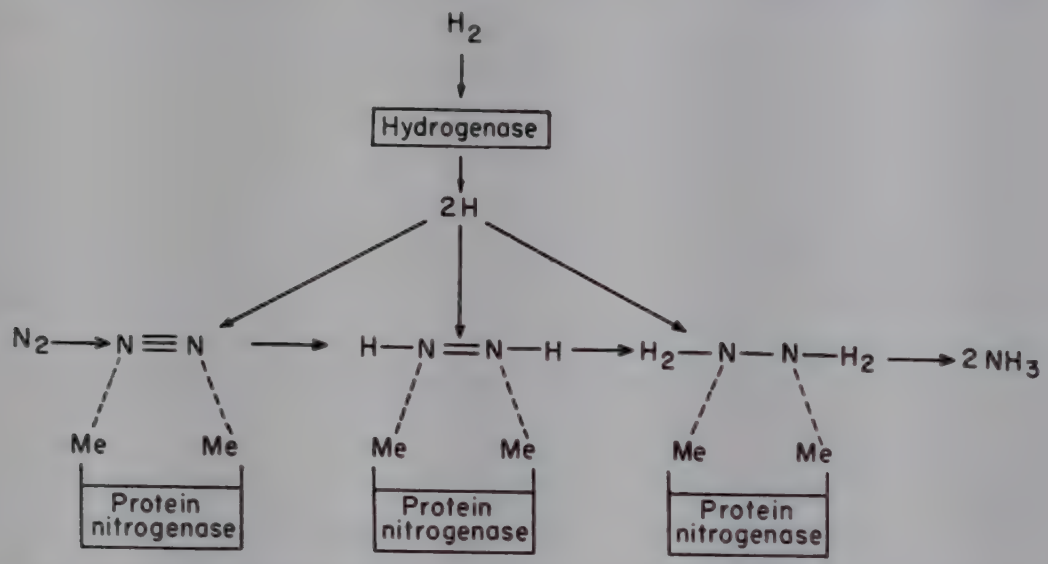


FIG. 3. Schematic diagram of nitrogenase and hydrogenase interactions in the nitrogen fixation process.

nomenon may be due to the fact that nitrogen would act competitively as an electron acceptor and perhaps also block a specific carrier involved in electron transport between the postulated pool of reducing power and the hydrogenase.

V. Biological Litho-oxidations of Secondary Importance

A. Oxidation of Iron

Ehrenberg (*198*) in 1836 first described microorganisms capable of oxidizing iron and attributed to them the natural formation of certain bog iron ores. The study of ferrobacteria was pursued by Winogradsky (*199*), Ellis (*200*), Molisch (*201*), Lieske (*202*), Cholodny (*203*), and many others. The extensive literature on the taxonomy and physiology of these organisms was reviewed in detail by Pringsheim (*204*), himself a major contributor to this field, and by van Beneden (*205*).

A first group of ferrobacteria, including in particular the classical forms *Sphaerotilus*, *Leptothrix*, and *Cladothrix*, is constituted by the Chlamydobacteriales or sheath bacteria. At the beginning of their morphological cycle, they are free, very mobile bacilli equipped with a single flagellum, which then divide in chains and surround themselves with a thick continuous sheath of polysaccharide nature and viscous consistence, forming very long filaments. The sheaths

gradually take on a brown-red color by incrustation of iron and manganese oxides. The filamentous ferrobacteria grow in sometimes considerable quantities in cold chalybeate springs or in water pipes where they may completely seal off the opening with their characteristic sticky, reddish mass. Pringsheim (*204*) took up critically the investigation of the many forms classified in distinct genera and species and showed that most of them are morphological variants belonging to only two different species, *Sphaerotilus discophorus* and S. *natans*.

The organism which was described by Ehrenberg (*198*) as *Gallionella* and which retained this generic name belongs to the Caulobacteriales or stalk bacteria. They are short, immobile, Gram-negative, curved inward like a comma or kidney bean. They attach themselves to solid surfaces by means of a coiled stalk which is secreted by their concave lateral edge and becomes impregnated progressively with a brown-red precipitate of ferric hydroxide.

The nutrition of the ferrobacteria has long been the subject of a controversy which continues to this day. Winogradsky (*199*) considered them to be chemo-lithotrophs which use the oxidation of ferrous to ferric iron as their sole energy source. However, Molisch (*201*) insisted that they could grow on peptone media in the presence or absence of iron, and classified them as heterotrophs in which the oxidation of iron is conditional and plays no metabolic role. Lieske (*202*) returned to the opinion of Winogradsky at least with regard to *Gallionella* and suggested that the development of this organism was based on the oxidation of ferrous carbonate by the following reaction:

$$4FeCO_3 + O_2 + 6H_2O = 4Fe(OH)_3 + 4CO_2 \qquad (-\Delta F^\circ_{298} = 40 \text{ kcal.})$$

Several authors tried to provide experimental evidence for the chemo-autotrophy of the ferrobacteria but these attempts, evaluated critically by Pringsheim (*204*), Cataldi (*206*), and Starkey (*207*), were not conclusive. In fact, most of the organisms were studied in impure cultures and on nonsynthetic media. Since the ferrobacteria grow near neutral pH levels and under these conditions iron is rapidly oxidized by the oxygen of the air, the culture media routinely contained proteins or humic acids, in order to stabilize ferrous iron, and it could not be excluded that growth was actually due to these organic components. Moreover, Starkey (*207*), on the basis of the feebly exergonic nature of the reaction, felt that the possibility of autotrophic development of the ferrobacteria was slim and that if it really existed, one might expect a 500 : 1 ratio by weight between iron oxidized and cellular matter synthesized.

However, Sartory and Mayer (*208*) have been able to isolate *Galli-*

onella in pure cultures on a strictly inorganic medium where the sole energy source was iron filings; therefore, it is most likely, as Lieske postulated, that at least this species is chemo-lithotrophic.

More recently, a new type of iron-oxidizing bacteria was discovered by Colmer and co-workers (*209*) in acid drainage waters from coal mines. These waters have a pH of about 3.0 and contain both free sulfuric acid and ferrous sulfate which is quite rapidly oxidized with the formation of large deposits of ferric hydroxide. Since the spontaneous oxidation of ferrous sulfate by oxygen is extremely slow at this pH, a biological process is obviously involved. These authors isolated from this natural medium a typical strain of *Thiobacillus thiooxidans* and another microorganism which they called *T. ferrooxidans*. The latter oxidizes thiosulfate and has the same morphological characteristics as the former but cannot oxidize elementary sulfur, while it utilizes the oxidation of ferrous to ferric iron as energy source. Growth can take place at pH 2.0–4.5, with an optimum at pH 3.5; in 3 days, the cultures oxidize up to 200 p.p.m. of ferrous iron, an amount which could not be oxidized spontaneously in air in less than two years. The chemolithotrophic character of *T. ferrooxidans* has been demonstrated (*210*). The growth balance sheet conformed to the prediction of Starkey who showed that the free energy efficiency could not rise above 3.5%. This value is lower than that of other autotrophic bacteria; this is due, in part, to the low energy level of iron oxidation and, in part, to the slight solubility of the carbon source, i.e., carbon dioxide, at the pH at which this bacterium can grow.

Leathen *et al.* (*211*) also isolated typical strains of *T. thiooxidans* and a bacterium which actively oxidizes iron from the same source, i.e., drainage water of coal mines. But this organism, in contrast to that of Colmer and associates (*209*), cannot oxidize thiosulfate or any other form of inorganic sulfur. Therefore it was not classed in the genus *Thiobacillus* but rather was called *Ferrobacillus ferrooxidans*.

It was first thought that the ferrous sulfate which is present in mine waters and constitutes the substrate of the ferrobacteria is derived from the oxidation of sulfide by sulfo-oxidizing bacteria, particularly by *T. thiooxidans* or the organism of Temple and Colmer which oxidizes both sulfur and iron. But Leathen *et al.* (*212*) found that the thiobacilli cannot oxidize pyrites (FeS_2), which are the inorganic compounds of sulfur in the coal deposits. These latter authors postulated that the pyrites are slowly oxidized in air by an entirely nonbiological process which leads to the formation of ferrous sulfate and free sulfuric acid:

$$2FeS_2 + 7O_2 + 2H_2O \rightarrow 2FeSO_4 + 2H_2SO_4$$

Ferrous sulfate in sulfuric acid medium is oxidized rapidly by the bacteria,

$$4FeSO_4 + 2H_2SO_4 + O_2 \rightarrow 2Fe_2(SO_4)_3 + 2H_2O$$

and the resultant ferric sulfate finally is hydrolyzed completely to ferric hydroxide. The oxidation of ferric sulfate accelerates the chemical oxidation of the pyrite by a mass action law effect and, moreover, $Fe_2(SO_4)_3$ catalyzes the oxidation of sulfuritic compounds; this explains why bacterial activity speeds the over-all process considerably.

B. Oxidation of Thiocyanate

Beijerinck (*213*) mentioned that enrichment cultures of thiobacilli degrade thiocyanate when this compound replaces thiosulfate in the medium. In 1937, Happold and Key (*214*) isolated a bacterium, later called *Thiobacillus thiocyanooxidans,* from the residual waters of gasworks and from sewage sludge. This organism, which was studied in detail by Happold *et al.* (*215*) and Youatt (*216*), is a strict aerobic autotroph. It can grow, like the other members of the genus *Thiobacillus,* by oxidizing elementary sulfur or thiosulfate, but it can also utilize thiocyanate as the sole source of energy, carbon, and nitrogen. In the latter case, its over-all metabolism corresponds to this equation:

$$CNS^- + 2O_2 + 2H_2O = SO_4^{2-} + NH_4 + CO_2 + 220 \text{ kcal.}$$

According to Youatt, this process comprises several successive steps. The first is the hydrolysis of thiocyanate to cyanate and sulfide:

$$CNS^- + H_2O \rightarrow HCNO + SH^-$$

Sulfide is then oxidized to sulfate by the same process as in other thiobacilli and cyanate is decomposed to carbon dioxide and ammonia by a second hydrolysis:

$$HCNO + H_2O \rightarrow CO_2 + NH_3$$

This last reaction yields the carbon and nitrogen sources the organism uses for growth. If the bacterium is grown on thiosulfate, carbonate and an ammonium salt must of course be added to provide a complete culture medium.

Sulfide is relatively toxic to *T. thiocyanooxidans* and its concentration in the medium may not rise above $1.6 \times 10^{-3}M$. Youatt elegantly demonstrated the oxidation of sulfide by growing the bacterium in a medium containing thioacetamide or thioacetate as substrate; these compounds are progressively hydrolyzed and yield sulfide without pushing its concentration beyond the inhibitory threshold. The observations of this author also seem to indicate that the enzymes responsible for

thiocyanate oxidation are clearly adaptive, while those involved in thiosulfate degradation are constitutive.

De Kruyff and co-workers (*217*) found that the ability to oxidize thiocyanate is not restricted to *T. thiocyanooxidans*. Under aerobic conditions thiocyanate is utilized as a substrate in place of thiosulfate by typical strains of *T. thioparus* and *T. denitrificans*, but not by *T. thiooxidans*. Moreover, the three species which can carry out this aerobic reaction can also grow anaerobically in the presence of thiocyanate and nitrate. Under these conditions, nitrate is reduced to N_2 by *T. denitrificans* but only to nitrite by the other two species. These recent observations suggest that there are no significant differences between *T. thiocyanooxidans* and *T. denitrificans*.

C. Oxidation of Methane

The oxidation of methane may be included into the group of biological litho-oxidations due to the fact that, from the biochemical point of view, CH_4 may be thought of not only as an organic compound—the first member of the series of paraffin hydrocarbons—but also as the product of the complete reduction of CO_2. This interpretation agrees with the demonstration by Barker and associates (*21*) of the biological formation of methane by the direct reduction of carbon dioxide rather than by the degradation of organic compounds of several carbon atoms. It also agrees with the fact that methane is used as the sole source of energy and carbon by microorganisms whose behavior resembles closely that of other chemo-lithotrophic bacteria.

The methane-oxidizing bacteria were discovered by Söhngen (*218*) in 1906. The species he described under the name of *Bacillus methanicus* is now known as *Methanomonas methanica* and has been studied in detail by Hutton and ZoBell (*219*) and Hutton (*220*). It is a short bacillus or coccobacillus, very mobile, Gram-negative, with a single polar flagellum. *M. methanica* is a strict aerobe and grows actively in an atmosphere containing methane and oxygen, forming a characteristic pellicle at the surface of liquid media.

The over-all oxidation of methane is summarized in this reaction:

$$CH_4 + 2O_2 = CO_2 + 2H_2O \qquad (-\Delta F^\circ_{298^\circ} = +207 \text{ kcal.})$$

It certainly comprises several successive steps but these have not yet been elucidated.

Slavnina (*221*) found that *M. methanica* can oxidize ethane and propane as well as methane. Hutton (*220*) characterized another species, *M. carbonatophila*, which differs primarily by the absence of a pellicle in liquid cultures.

Methane-oxidizing bacteria are widely distributed in the soil where they oxidize the methane derived from the degradation of cellulose by methane-forming bacteria and, occasionally, the emanations of methane coming from deep-lying hydrocarbon deposits. Counts of methane-oxidizing bacteria have been used for geomicrobiological detection of petroleum deposits (*222*).

D. Oxidation of Carbon Monoxide

Beijerinck and van Delden (*223*) are generally credited with the discovery of a bacterium capable of oxidizing carbon monoxide aerobically by this reaction:

$$CO + \tfrac{1}{2}O_2 = CO_2 \qquad (-\Delta F^\circ_{298^\circ} = +66 \text{ kcal.})$$

They described a Gram-positive, immobile bacterium which they called *Bacillus oligocarbophilus,* but, as emphasized by Kistner (*224*) in his critical survey of the literature, they did not bring formal proof of its ability to oxidize CO. Later Kaserer (*128*), during his studies on the bacterial oxidation of H_2, isolated a bacterium which he deemed capable of CO oxidation and identical with the organism found by Beijerink. Lantzsch (*225*) gave a more detailed morphological description of this organism and retained the name of *B. oligocarbophilus* although Bisset and Grace (*62*) felt that some of its reported characteristics, such as Gram-positive staining, low resistance to acid, and the transitory filamentous phase, corresponded more closely to a yeast, like *Nocardia,* than to a bacterial species.

Hasemann (*226*) was the first to provide definite proof of bacterial oxidation of CO by obtaining from enrichment cultures in inorganic media and in the presence of illuminating gas several pure strains of microorganisms which consume carbon monoxide aerobically. More recently, Jones and Scott (*227*) demonstrated rapid disappearance of CO from the atmosphere of a coal mine and were able to relate this purification of the air clearly to the activity of bacteria. Finally, Kistner (*224*) obtained a pure culture from sewage sludge of a mobile, Gram-negative, monotrichous bacterium which can grow aerobically by oxidizing either CO or molecular hydrogen or various organic substrates. Its morphological and physiological characteristics are very different from those of *B. oligocarbophilus* but similar to those of the hydrogen bacteria, and it was therefore named *Hydrogenomonas carboxyvorans.* The hydrogenase of this facultative chemo-lithotroph as well as the enzymes which catalyze the oxidation of CO are both adaptive enzymatic systems, formed only when the cells are grown in the presence of the respective substrates.

Anaerobic bacteria can also metabolize carbon monoxide. Kluyver and Schnellen (228) showed that the strict anaerobic methane-forming *Methanobacterium barkerii* can produce methane from CO and H_2:

$$CO + 3H_2 \rightarrow CH_4 + H_2O$$

This bacterium also produces methane in the absence of H_2, in an atmosphere containing a mixture of CO and nitrogen or 100% CO. Under these conditions, where the energy source is an organic substrate, significant amounts of CO_2 are produced during the development of the cultures and if this is absorbed by alkali the accumulation of appreciable quantities of H_2 in the gaseous phase is noted. These observations suggest a mechanism involving two successive reactions. The first is the hydrolytic oxidation of CO with the formation of CO_2 and H_2, and the second is the hydrogenation of CO_2 by molecular hydrogen in the hydrogenase reaction which is also carried out by the other methane bacteria. The entire reaction sequence is as follows:

$$\begin{aligned} 4CO + 4H_2O &\rightarrow 4CO_2 + 4H_2 \\ CO_2 + 4H_2 &\rightarrow CH_4 + 2H_2O \\ \hline 4CO + 2H_2O &\rightarrow 3CO_2 + CH_4 \end{aligned}$$

M. formicicium behaves in an analogous manner but tolerates only lower partial pressures of CO than *M. barkerii*. That carbon monoxide is initially oxidized to CO_2 and is not, as might be thought, an intermediate in the reduction of CO_2 to methane is shown by the behavior of *M. omelianskii*, which produces CH_4 from H_2 and CO_2 but not from CO.

Recently, Yagi (229) showed that cell-free extracts of *Desulfovibrio desulfuricans* oxidize carbon monoxide to CO_2 anaerobically by a process coupled with the reduction of sulfite to sulfide:

$$3CO + H_2SO_3 \rightarrow 3CO_2 + H_2S$$

In this instance, as in that of methane formation by *M. barkerii*, it seems that the first reaction step involves the hydrolytic oxidation of CO to CO_2 and H_2. At the expense of the molecular hydrogen thus produced, sulfite is reduced secondarily with the participation of hydrogenase and the sulfite reductase enzyme system.

References

1. M. Stephenson, "Bacterial Metabolism," 3rd ed., p. 241. Longmans, Green, London, 1949.
2. W. W. Umbreit, *Bacteriol. Revs.* **11**, 157 (1947).
3. C. B. van Niel, *Ann. Rev. Microbiol.* **8**, 105 (1954).
4. H. Lees, "Biochemistry of Autotrophic Bacteria." Butterworths, London, 1955.

5. J. W. Foster, *in* "Bacterial Physiology" (C. H. Werkman and P. W. Wilson, eds.), p. 361. Academic Press, New York, 1951.
6. Autotrophic Micro-Organisms, *4th Symposium Soc. Gen. Microbiol.* (1954).
7. G. N. Lewis and M. Randall, "Thermodynamics and the Free Energy of Chemical Substances." McGraw-Hill, New York, 1923.
8. R. Wurmser, "Oxydations et Réductions." Presses Univ. France, Paris, 1930.
9. C. B. van Niel, *Arch. Mikrobiol.* **7,** 323 (1936).
10. L. G. M. Baas-Becking and G. S. Parks, *Physiol. Revs.* **7,** 85 (1927).
11. E. F. Terroine and R. Wurmser, *Bull. soc. chim. biol.* **4,** 519 (1922).
12. S. Winogradsky, *Ann. Inst. Pasteur* **4,** 213 (1890).
13. O. Meyerhof, *Pflügers Arch. ges. Physiol.* **164,** 353 (1916).
14. H. Lees, *4th Symposium Soc. Gen. Microbiol.* p. 84 (1954).
15. S. Winogradsky, "Beiträge zur Morphologie und Physiologie der Bakterien." Vol. I. Arthur Felix, Leipzig, 1888.
16. T. W. Engelmann, *Botan. Ztg.* **40,** 185 (1882).
17. C. B. van Niel, *Arch. Mikrobiol.* **3,** 1 (1931); *in* "Photosynthesis in Plants" (J. Frank and W. E. Loomis, eds.), p. 437. Iowa State College Press, Ames, Iowa, 1949.
18. H. Larsen, *4th Symposium Soc. Gen. Microbiol.* p. 186 (1954).
19. J. Buder, *Jahrb. wiss. Botan.* **58,** 525 (1919).
20. H. F. Blum, *Am. Naturalist* **71,** 350 (1937).
21. H. A. Barker, S. Ruben, and M. D. Kamen, *Proc. Natl. Acad. Sci. U. S.* **26,** 426 (1940).
22. D. D. Woods and J. Lascelles, *4th Symposium Soc. Gen. Microbiol.* p. 1 (1954).
23. M. F. Utter and H. G. Wood, *Advances in Enzymol.* **12,** 41 (1951).
24. C. H. Werkman, *in* "Bacterial Physiology" (C. H. Werkman and P. W. Wilson, eds.), p. 404. Academic Press, New York, 1951.
25. S. Ochoa, *Physiol. Revs.* **31,** 56 (1951).
26. S. Ochoa, A. H. Mehler, and A. Kornberg, *J. Biol. Chem.* **174,** 979 (1948).
27. M. F. Utter, K. Kurahashi, and I. A. Rose, *J. Biol. Chem.* **207,** 803 (1954).
28. S. H. Pomerantz, *Federation Proc.* **17,** 290 (1958).
29. J. P. Aubert, G. Milhaud, and J. Millet, *Ann. Inst. Pasteur* **92,** 516 (1957).
30. P. A. Trudinger, *Biochem. J.* **64,** 274 (1956).
31. F. H. Bergmann, J. C. Towne, and R. H. Burris, *J. Biol. Chem.* **230,** 13 (1958).
32. B. A. McFadden, *J. Bacteriol.* **77,** 339 (1959).
33. J. Judis, H. Koffler, and D. M. Powelson, *Proc. Soc. Exptl. Biol. Med.* **89,** 665 (1955).
34. I. Suzuki and C. H. Werkman, *Bacteriol. Proc.* p. 120 (1957).
35. K. G. Vogler and W. W. Umbreit, *J. Gen. Physiol.* **26,** 157 (1942).
36. K. Baalsrud and K. S. Baalsrud, *Arch. Mikrobiol.* **20,** 34 (1954).
37. R. W. Newburgh, *J. Bacteriol.* **68,** 93 (1954).
38. W. Vishniac and M. Santer, *Bacteriol. Revs.* **21,** 195 (1957).
39. B. Skarzynski and W. Ostrowski, *Nature* **182,** 933 (1958).
40. S. Winogradsky and V. Omelianskii, *Centr. Bakteriol.* **5,** 329, 377, and 429 (1899).
41. O. Meyerhof, *Pflügers Arch. ges. Physiol.* **166,** 240 (1916).
42. H. L. Jensen, *Nature* **165,** 974 (1950).
43. T. Y. Kingma-Boltjes, *Arch. Mikrobiol.* **6,** 79 (1935).

44. E. B. Fred and A. Davenport, *Soil. Sci.* **11,** 389 (1921).
45. T. J. Murray, *Proc. Soc. Exptl. Biol. Med.* **20,** 301 (1923).
46. J. W. Hes, Thesis, University of Groningen (1937).
47. H. Lees, *Biochem. J.* **52,** 134 (1952).
48. J. H. Quastel and P. G. Scholefield, *Bacteriol. Revs.* **15,** 1 (1951).
49. W. O. Kermak and H. Lees, *Sci. Progr.* **40,** 44 (1952).
50. H. L. Jensen and H. Sörensen, *Acta Agr. Scand.* **2,** 295 (1952).
51. R. L. Starkey, *Soil Sci.* **39,** 197 (1935).
52. M. V. Tyulpanova-Mosevich, *Arch. Sci. Biol.* (*U.S.S.R.*) **30,** 203 (1930).
53. G. N. Cohen and J. Monod, *Bacteriol. Revs.* **21,** 169 (1957).
54. S. C. Rittenberg and R. P. Grady, *J. Bacteriol.* **60,** 509 (1950).
55. B. Niklevskii, *Jahrb. wiss. Botan.* **48,** 113 (1910).
56. W. Ruhland, *Jahrb. wiss. Botan.* **63,** 321 (1924).
57. A. J. Kluyver and A. Manten, *Antonie van Leeuwenhoek J.* **8,** 71 (1948).
58. E. Wilson, H. A. Stout, D. Powelson, and H. Koffler, *J. Bacteriol.* **65,** 283 (1953).
59. J. H. Schloesing and C. A. Muntz, *Compt. rend. acad. sci.* **84,** 301; **85,** 1018; **86,** 891; **88,** 1074 (1877–1879).
60. S. Winogradsky, *Ann. Inst. Pasteur* **4,** 213 (1890); **5,** 577 (1891).
61. R. S. Breed, E. G. D. Murray, A. P. Hitchens, and others, "Bergey's Manual of Determinative Bacteriology," 6th ed. Williams & Wilkins, Baltimore, Maryland, 1948.
62. K. A. Bisset and J. B. Grace, *4th Symposium Soc. Gen. Microbiol.* p. 28 (1954).
63. D. H. Nelson, *Centr. Bakteriol.* **83,** 287 (1931).
64. J. Meiklejohn, *4th Symposium Soc. Gen. Microbiol.* p. 68 (1954).
65. D. W. Cutler and L. M. Crump, *Ann. Appl. Biol.* **20,** 291 (1933).
66. J. H. Quastel, P. G. Scholefield and J. W. Stevenson, *Nature* **166,** 940 (1950).
67. H. L. Jensen, *J. Gen. Microbiol.* **5,** 360 (1951).
68. H. D. Isenberg, A. Schatz and S. H. Hutner, *Proc. Soc. Am. Bacteriol.* **52,** 152 (1952).
69. E. L. Schmidt, *Science* **119,** 187 (1954).
70. O. R. Eylar and E. L. Schmidt, *J. Gen. Microbiol.* **20,** 473 (1959).
71. T. Fischer, E. Fischer, and M. D. Appleman, *J. Gen. Microbiol.* **14,** 238 (1956).
72. C. C. Delwiche, *in* "Inorganic Nitrogen Metabolism" (W. D. McElroy and B. Glass, eds.), p. 218. Johns Hopkins, Baltimore, 1956.
73. A. Nason and H. Takahashi, *Ann. Rev. Microbiol.* **12,** 203 (1958).
74. A. J. Kluyver and H. J. L. Donker, *Chem. Zelle u. Gewebe* **13,** 134 (1926).
75. H. Lees, *Nature* **169,** 156 (1952).
76. T. Hofman and H. Lees, *Biochem. J.* **54,** 579 (1953).
77. T. Hofman and H. Lees, *Biochem. J.* **52,** xix (1952).
78. A. A. Imshenetskii, E. L. Ruban, and O. D. Buzina, *Mikrobiologyia* **24,** 539 (1955); **25,** 1245 (1956).
79. D. Spencer, H. Takahashi, and A. Nason, *J. Bacteriol.* **73,** 533 (1957).
80. M. Zucker and A. Nason, *J. Biol. Chem.* **213,** 463 (1955).
81. R. Klausmeier and R. C. Bard, *J. Bacteriol.* **68,** 129 (1954).
82. G. G. Roussos, H. Takahashi, and A. Nason, *J. Bacteriol.* **73,** 594 (1957).
83. H. Lees and J. R. Simpson, *Biochem. J.* **65,** 297 (1957).
84. W. D. Butt and H. Lees, *Nature* **182,** 732 (1958).

85. G. A. Zavarzin, *Doklady Akad. Nauk. S.S.S.R.* **113,** 1361 (1957).
86. K. Baalsrud and K. S. Baalsrud, *in* "Phosphorus Metabolism." (W. D. McElroy and B. Glass, eds.), Vol. 2, p. 544. Johns Hopkins, Baltimore, 1952.
87. G. W. Harmsen, *Chem. Weekblad* **35,** 495 (1938).
88. C. D. Parker, *Nature* **159,** 439 (1947).
89. K. Baalsrud, *4th Symposium Soc. Gen. Microbiol.* p. 54 (1954).
90. S. Winogradsky, *Ann. Inst. Pasteur* **1,** 548 (1887).
91. F. Keil, *Beitr. Biol. Pflanz.* **2,** 335 (1912).
92. M. S. Cataldi, *Rev. inst. bacteriol. (Buenos Aires)* **9,** 393 (1940).
93. H. Larsen, *Kgl. Norske Videnskab. Selskabs. Skrifter* **1,** (1953).
94. S. R. Elsden, *4th Symposium Soc. Gen. Microbiol.* p. 202 (1954).
95. G. Guittoneau, *Compt. rend. acad. sci.* **181,** 261 (1925).
96. R. L. Starkey, *Soil Sci.* **39,** 197 (1935).
97. R. Sijderius, Thesis, Amsterdam (1946).
98. K. G. Vogler and W. W. Umbreit, *Soil Sci.* **51,** 331 (1941).
99. W. W. Umbreit and T. F. Anderson, *J. Bacteriol.* **44,** 317 (1942).
100. R. L. Starkey, *J. Bacteriol.* **33,** 545 (1937).
101. G. Guittoneau and J. Keilling, *Ann. Agron.* [N.S.] **2,** 690 (1932).
102. C. Fromageot, *Advances in Enzymol.* **7,** 369 (1947).
103. W. Vishniac, *J. Bacteriol.* **64,** 363 (1952).
104. P. A. Trudinger, *Biochim. et Biophys. Acta* **31,** 270 (1959).
105. W. Ostrowski and A. Krawczyk, *Acta Biochim. Polon.* **4,** 249 (1957).
106. R. L. Starkey, *J. Bacteriol.* **28,** 365 (1934).
107. H. Gleen and J. H. Quastel, *Appl. Microbiol.* **1,** 70 (1953).
108. H. Tamiya, H. Haga, and H. Huzisige, *Acta Phytochim. (Japan)* **12,** 173 **(1941).**
109. J. W. Newton and M. D. Kamen, *Biochim. et Biophys. Acta* **21,** 71 (1956).
110. M. D. Kamen and L. P. Vernon, *J. Bacteriol.* **67,** 617 (1954).
111. J. Gibson and H. Larsen, *Biochem. J.* **60,** xxvii (1955).
112. R. Klimer, B. Skarzynski, and T. W. Szczepkowski, *Acta Biochim. Polon.* **3,** 261 (1956).
113. P. A. Trudinger, *Biochim. et Biophys. Acta* **30,** 211 (1958).
114. J. P. Aubert, G. Milhaud, C. Moncel, and J. Millet, *Compt. rend. acad. sci.* **246,** 1616 (1958).
115. G. Milhaud, J. P. Aubert, and J. Millet, *Compt. rend. acad. sci.* **246,** 1766 (1958).
116. G. Milhaud, J. P. Aubert, and J. Millet, *Compt. rend. acad. sci.* **244,** 1289 (1957).
117. G. Knaysi, *J. Bacteriol.* **46,** 451 (1943).
118. H. A. Barker and A. Kornberg, *J. Bacteriol.* **68,** 655 (1954).
119. M. Santer and W. Vishniac, *Bacteriol. Proc.* p. 131 (1958).
120. H. Margulies and M. Santer, *Bacteriol. Proc.* p. 121 (1958).
121. P. W. Robbins and F. Lipmann, *J. Am. Chem. Soc.* **78,** 6409 (1956).
122. L. G. Wilson and R. S. Bandurski, *J. Am. Chem. Soc.* **80,** 5576 (1958).
123. H. D. Peck, Jr., *Proc. Natl. Acad. Sci. U. S.* **45,** 701 (1959).
124. J. S. Fruton and S. Simmonds, "General Biochemistry," 2nd ed. Wiley, New York, 1958.
125. C. Fromageot, *in* "The Enzymes" (J. B. Sumner and K. Myrback, eds.), Vol. II, Part 1, p. 609. Academic Press, New York, 1951.
126. L. Fridovich and P. Handler, *J. Biol. Chem.* **221,** 323 (1956).

127. H. Gest, *Bacteriol. Revs.* **18,** 43 (1954).
128. H. Kaserer, *Centr. Bakteriol.* **II 16,** 681 (1906).
129. B. Niklevskii, *Jahrb. wiss. Botan.* **48,** 111 (1910).
130. A. Schatz and C. Bovell, *J. Bacteriol.* **63,** 87 (1952).
131. M. W. Beijerinck, *Centr. Bakteriol.* **II 1,** 1 (1895).
132. J. K. Baars, Thesis, Delft (1930).
133. M. Stephenson and L. H. Stickland, *Biochem. J.* **25,** 215 (1931).
134. J. R. Postgate, *J. Gen. Microbiol.* **15,** 186 (1956).
135. R. L. Starkey and K. M. Wight, *Am. Gas Assoc. Tech. Rept.* No. 1945 (1945).
136. K. R. Butlin and M. Adams, *Nature* **160,** 154 (1947).
137. J. C. Senez and B. E. Volcani, *Compt. rend. acad. sci.* **232,** 1035 (1951).
138. H. Gest, *J. Bacteriol.* **63,** 111 (1952).
139. H. Gest and M. D. Kamen, *Science* **109,** 558 (1949).
140. H. Gaffron, *Am. J. Botan.* **27,** 273 (1940).
141. A. W. Frenkel, *Arch. Biochem. Biophys.* **38,** 219 (1952).
142. A. W. Frenkel and C. Rieger, *Nature* **167,** 1030 (1951).
143. H. Gest, *Bacteriol. Revs.* **15,** 183 (1951).
144. A. I. Krasna and D. Rittenberg, *Proc. Natl. Acad. Sci. U. S.* **40,** 225 (1954).
145. H. J. Koepsell and M. J. Johnson, *J. Biol. Chem.* **145,** 379 (1942).
146. L. E. Mortenson and P. W. Wilson, *J. Bacteriol.* **62,** 513 (1951).
147. J. Wilson, L. O. Krampitz, and C. H. Werkman, *Biochem. J.* **42,** 598 (1948).
148. H. D. Peck, Jr. and H. Gest, *J. Bacteriol.* **73,** 569 (1957).
149. J. C. Senez, *Bull. soc. chim. biol.* **36,** 541 (1954).
150. J. Millet, *Compt. rend. acad. sci.* **238,** 408 (1954).
151. M. Stephenson and L. H. Stickland, *Biochem. J.* **26,** 712 (1932).
152. D. D. Woods, *Biochem. J.* **30,** 515 (1936).
153. E. J. Ordal and H. O. Halvorson, *J. Bacteriol.* **38,** 199 (1939).
154. H. D. Peck and H. Gest, *J. Bacteriol.* **73,** 706 (1957).
155. M. Green and P. W. Wilson, *J. Bacteriol.* **65,** 511 (1953).
156. H. Wieland and H. J. Pistor, *Ann.* **522,** 116 (1936).
157. S. Yamagata and H. Nakamura, *Acta Phytochim. (Japan)* **10,** 297 (1938).
158. G. N. Smith and R. O. Marshall, *Arch. Biochem. Biophys.* **39,** 395 (1952).
159. M. Stephenson and L. H. Stickland, *Biochem. J.* **25,** 205 (1931).
160. L. Michaelis and E. S. Hill, *J. Gen. Physiol.* **16,** 859 (1933).
161. H. D. Peck and H. Gest, *J. Bacteriol.* **71,** 70 (1956).
162. W. Curtis and E. J. Ordal, *J. Bacteriol.* **68,** 351 (1954).
163. A. Farkas, L. Farkas, and J. Yudkin, *Proc. Roy. Soc.* **B115,** 373 (1934).
164. H. D. Hoberman and D. Rittenberg, *J. Biol. Chem.* **147,** 211 (1943).
165. A. I. Krasna and D. Rittenberg, *J. Am. Chem. Soc.* **76,** 3015 (1954).
166. A. I. Krasna and D. Rittenberg, *Proc. Natl. Acad. Sci. U. S.* **42,** 180 (1956).
167. H. D. Peck, Jr., A. San Pietro and H. Gest, *Proc. Natl. Acad. Sci. U. S.* **42,** 13 (1956).
168. M. Bovarnik, *Proc. Soc. Exptl. Biol. Med.* **47,** 191 (1941).
169. M. Green and P. W. Wilson, *J. Bacteriol.* **65,** 511 (1953).
170. D. E. Atkinson and B. A. McFadden, *J. Biol. Chem.* **210,** 885 (1954).
171. A. I. Krasna and D. Rittenberg, *Biochim. et Biophys. Acta* **26,** 526 (1957).
172. A. L. Shug, P. B. Hamilton, and P. W. Wilson, *in* "Inorganic Nitrogen Metabolism" (W. D. McElroy and B. Glass, eds.), p. 344. Johns Hopkins, Baltimore, 1956.

173. J. C. Sadana and V. Jagannathan, *Biochim. et Biophys. Acta* **19**, 440 (1956).
174. Y. Kondo, T. Kameyama, and N. Tamiya, *J. Biochem. (Tokyo)* **44**, 61 (1957).
175. H. F. Fischer, A. L. Krauss, and D. Rittenberg, *J. Biol. Chem.* **209**, 7 (1954).
176. H. R. Whiteley and E. J. Ordal, in "Inorganic Nitrogen Metabolism" (W. D. McElroy and B. Glass, eds.), p. 521. Johns Hopkins, Baltimore, 1956.
177. W. S. Waring and C. H. Werkman, *Arch. Biochem.* **4**, 75 (1944).
178. M. E. Winfield, *Rev. Pure and Appl. Chem. (Australia)* **5**, 217 (1955).
179. H. B. Mahler, A. S. Fairhurst, and B. Mackler, *J. Am. Chem. Soc.* **77**, 1 (1955).
180. D. E. Green and L. H. Stickland, *Biochem. J.* **28**, 898 (1934).
181. B. Mackler, H. R. Mahler, and D. E. Green, *J. Biol. Chem.* **210**, (1954).
182. S. Korkes, in "Phosphorus Metabolism" (W. D. McElroy and B. Glass, ed Vol. 2, p. 502. Johns Hopkins, Baltimore, 1952.
183. H. D. Peck, Jr. and H. Gest, *Biochim. et Biophys. Acta* **15**, 387 (1954).
184. L. Packer and W. Vishniac, *Bacteriol. Proc.* p. 111 (1954).
185. C. L. Wittenberger and R. Repaske, *Bacteriol. Proc.* p. 106 (1958).
186. W. K. Joklik, *Australian J. Exptl. Med. Sci.* **28**, 321 (1950).
187. J. C. Senez and F. Pichinoty, *Biochim. et Biophys. Acta* **27**, 569; **28**, (1958).
188. S. C. Kinsky, *J. Biol. Chem.* **234**, 973 (1959).
189. L. F. Vernon and M. D. Kamen, *J. Biol. Chem.* **211**, 643, 663 (1954).
190. A. Tissieres, *Biochem. J.* **64**, 582 (1956).
191. S. B. Lee, and P. W. Wilson, *J. Bact. Chem.* **151**, 377 (1943).
192. C. E. Fogg and M. Wolfe, *4th Symposium Soc. Gen. Microbiol.* (1954).
193. S. Hiai, T. Mori, S. Hino, and T. Mori, *J. Biochem. (Tokyo)* **44**, 839 (19
194. E. S. Lindstrom, R. H. Burris, and P. W. Wilson, *J. Bacteriol.* **58**, (1949).
195. H. Gest, I. Judis, and H. D. Peck, Jr., in "Inorganic Nitrogen Metabolism D. McElroy and B. Glass eds.), p. 268. Johns Hopkins, Baltimore, 1956.
196. J. Le Gall, J. C. Senez, and F. Pichinoty, *Ann. Inst. Pasteur* **96**, 223 (19
197. P. B. Hamilton, W. E. Magee, and L. E. Mortenson, *Bacteriol. Proc.* p (1953).
198. L. C. Ehrenberg, *Ann. Physik (Leipzig)* **38**, 213 (1836).
199. S. Winogradsky, *Botan. Z.* **46**, 262 (1888); *Centr. Bakteriol.* **II 57**, 1 (19
200. D. Ellis, *Centr. Bakteriol.* **II 19**, 502 (1907).
201. H. Molisch, "Die Eisenbakterien" Fischer, Jena, 1910.
202. R. Lieske, *Jahrb. wiss. Botan.* **49**, 91 (1911).
203. N. Chalilov, "Die Eisenbakterien." Fischer, Jena, 1928.
204. E. G. Pringsheim, *Phil. Trans. Roy. Soc.* **B233**, 453 (1949); *Biol. Revs* **200** (1949).
205. G. van Beneden, *Hydrobiologia* **3**, 1 (1951).
206. M. S. Cataldi, *Folia Biol. Buenos Aires* p. 237 (1937).
207. R. L. Starkey, *Science* **102**, 532 (1945).
208. A. Sartory and J. Mayer, *Compt. rend. acad. sci.* **223**, 541 (1947).
209. A. R. Colmer, K. L. Temple, and M. E. Hinkle, *J. Bacteriol.* **59**, 317 (1
210. K. L. Temple and A. R. Colmer, *J. Bacteriol.* **62**, 605 (1951).
211. W. W. Loutfee, N. A. Kuisel, and S. A. Bailey, *J. Bacteriol.* **72**, 700 (1
212. W. W. Loutfee, S. A. Bailey, and L. D. McIntyre, *Appl. Microbiol.* **1**, 6 (1953).

13. M. W. Beijerinck, *Centr. Bakteriol.* **II 11,** 593 (1904).
14. F. C. Happold and A. Key, *Biochem. J.* **31,** 1323 (1937).
15. F. C. Happold, K. I. Johnstone, H. J. Rogers, and J. B. Youatt, *J. Gen. Microbiol.* **10,** 261 (1954).
16. J. B. Youatt, *J. Gen. Microbiol.* **11,** 139 (1954).
17. C. D. de Kruyff, J. P. Van der Walt, and H. M. Schwarz, *Antonie van Leeuwenhoek* **23,** 305 (1957).
18. N. L. Sohngen, *Centr. Bakteriol.* **II 15,** 513 (1906).
19. W. E. Hutton, and C. E. ZoBell, *J. Bacteriol.* **58,** 463 (1949).
20. W. E. Hutton, Thesis, Univ. of California, Los Angeles (1953).
21. G. P. Slavnina, *Mikrobiologiya* **17,** 76 (1947).
22. E. Beerstecher, Jr., "Petroleum Microbiology." Elsevier, Amsterdam, 1954.
23. M. W. Beijerinck and A. van Delden, *Centr. Bakteriol.* **II 11,** 593 (1904).
24. A. Kistner, *Proc. Koninkl. Ned. Akad. Wetenschap.* **C56,** 443 (1953); **C57,** 186 (1954).
25. K. Lantzsch, *Centr. Bakteriol.* **II 57,** 369 (1922).
26. W. Hasemann, *Biochem. Z.* **184,** 147 (1927).
27. C. W. Jones and C. S. Scott, *Ind. Eng. Chem.* **31,** 775 (1939).
28. A. J. Kluyver and C. C. T. P. Schnellen, *Arch. Biochem* **14,** 57 (1947).
29. T. Yagi, *Biochim. et Biophys. Acta* **30,** 194 (1958).

CHAPTER 9

Comparative Biochemistry of Glycolysis

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Chemical Reactions Involved in Glycolysis*

The conversion of glucose or glycogen to lactic acid in living cells or ...es is known under the term of glycolysis. The sequence of reactions ...lved in this process were uncovered largely through the work of ...oden, Meyerhof, and Cori. These reactions and the enzymes cata...ng them are outlined in Table I. Glycolysis proceeds without par...ation of oxygen; DPN$^+$ is reduced during the oxidation of glyc...dehyde-3-phosphate (see Table I, Reaction 6); DPNH, in turn, is ...d for the reduction of pyruvate to lactate (Reaction 11) and in this ...ner DPN$^+$ is regenerated. During the glycolysis of 1 mole of glucose,

* In this chapter the following abbreviations are used: DPN$^+$, diphosphopyridine ...otide; DPNH, reduced diphosphopyridine nucleotide; TPN$^+$, triphosphopyridine ...otide; TPNH, reduced triphosphopyridine nucleotide; ATP, adenosinetriphos...e; ADP, adenosine diphosphate; P_i, inorganic phosphate; ATPase, adeno...triphosphatase; HDP, fructose-1,6-diphosphate.

TABLE I

REACTIONS AND ENZYMES INVOLVED IN THE EMBDEN-MEYERHOF SCHEME OF GLYCOLYSIS

Reaction	Substrate(s)		Product(s)	Enzyme
1	Glucose + ATP	⟶	Glucose-6-phosphate + ADP	Hexokinase
1a	Glycogen + P_i	⟷	Glucose-1-phosphate	Phosphorylase
1b	Glucose-1-phosphate	⟷	Glucose-6-phosphate	Phosphoglucomutase
2	Glucose-6-phosphate	⟷	Fructose-6-phosphate	Phosphoglucose isomerase
3	Fructose-6-phosphate + ATP	⟶	Fructose-1.6-diphosphate + ADP	Phosphofructokinase
4	Fructose-1,6-diphosphate	⟷	Glyceraldehyde-3-phosphate + dihydroxyacetone-1-phosphate	Aldolase
5	Dihydroxyacetone-1-phosphate	⟷	Glyceraldehyde-3-phosphate	Triosephosphate isomerase
6	Glyceraldehyde-3-phosphate + P_i + DPN^+	⟷	1,3-diphosphoglycerate + DPNH	Glyceraldehyde-3-phosphate dehydrogenase
7	1,3-Diphosphoglycerate + ADP	⟷	3-phosphoglycerate + ATP	Phosphoglyceric phosphokinase
8	3-Phosphoglycerate	⟷	2-Phosphoglycerate	Phosphoglyceric mutase
9	2-Phosphoglycerate	⟷	Phosphoenol pyruvate	Enolase
10	Phosphoenol pyruvate + ADP	⟷	Pyruvate + ATP	Pyruvate kinase
11	Pyruvate + DPNH	⟷	Lactate + DPN^+	Lactic dehydrogenase

ATP balance:

Glycolysis of glucose

2 ATP ⟶ 2 ADP	(Reactions 1 and 3)
4 ADP ⟶ 4 ATP	(Reactions 7 and 10)
Net balance: + 2 ATP	

Glycolysis of glycogen

1 ATP ⟶ 1 ADP	
4 ATP ⟶ 4 ADP	
Net balance: + 3 ATP	

2 P_i: ⟶ 1,3 Diphosphoglyceric acid	(Reaction 6)
2 DPN^+ ⟶ 2 DPNH	(Reaction 6)
2 DPNH ⟶ 2 DPN^+	(Reaction 11)

2 moles of ATP are used for the formation of HDP (Reactions 1 and 3) while 4 moles of ADP are converted to ATP (Reactions 7 and 10), resulting in a net gain of 2 moles of ATP. For the glycolysis of glycogen only 1 mole of ATP is required with a net gain of 3 moles of ATP. Thus, energy-rich phosphate bonds are generated during glycolysis. These energy relationships and balances have been discussed by Krebs and Kornberg (*1*). The energy yield from glycolysis is low when compared with that produced by the oxidation of glucose. Glycolysis results not only in a net gain of ATP, but also in a corresponding loss of inorganic phosphate. The effects of these changes on the regulation and on the rate of glycolysis in various tissues and cells will be considered below (Sections II, B and II, C).

In the glycolysis of glycogen to lactic acid every reaction is reversible with the exception of the conversion of fructose-6-phosphate to fructose-1,6-diphosphate (Reaction 3). The occurrence in certain tissues and cells of a specific fructose-1,6-diphosphatase (*2–4*), catalyzing the dephosphorylation of fructose-1,6-diphosphate to fructose-6-phosphate and inorganic phosphate, affords an opportunity for the synthesis of glycogen from lactate, or from other intermediates of glycolysis, through a reversal of the reactions of the Embden-Meyerhof scheme. Another irreversible reaction of glycolysis is the phosphorylation of glucose, catalyzed by hexokinase. However, glucose can be formed from glucose-6-phosphate, produced either from glycogen or by a reversal of the glycolytic reactions, through the action of a specific glucose-6-phosphatase (*5, 6*).

A variety of factors may affect glycolysis of a particular tissue or cell. If any one of them should become limiting, this would determine the rate at which glucose is converted to lactic acid. Some of these factors will be discussed below.

II. Regulatory Factors

A. Glucose Transport into the Cell

An important source of intracellular glucose is provided by the transport of this hexose. The passage of glucose into the cell from its surrounding medium proceeds at a faster rate than can be accounted for by simple diffusion; thus, a mechanism for the active transport of glucose must exist. Although the process is rapid, the intracellular concentration in many tissues and cells is extremely low. In these cells the capacity of the glycolytic enzymes to utilize glucose is higher than the rate at which this hexose is entering the cell. This phenomenon is probably the basis for the observation that the rate of glycolysis in cell-free

extracts frequently is higher than in the intact cell. It appears that glucose transport in certain mammalian tissues is subject to hormonal control. For example, it has been shown that insulin increases glucose transport into skeletal and heart muscles (*7–16*) while it has no effect on this process in the red cell or in the brain (*9, 10*). Conversely, a hormone of the anterior pituitary or a metabolic product thereof inhibits glucose transport (*17*). Glucose transport into red cells is inhibited also by phlorizin (*18*), and into schistosomes (*19*), as well as into red cells (*20*), by alkyldibenzylamines.

B. ADP-ATP Ratios

During glycolysis of glucose, twice as much ATP is produced than is utilized (see ATP balance, Table I). Glycolysis cannot occur unless ADP is supplied to serve as a phosphate acceptor from 1,3-diphosphoglycerate (Reaction 7) and from phosphoenolyruvate (Reaction 10). The action of various ATPases which catalyze the irreversible hydrolysis of ATP to ADP and inorganic phosphate represents one type of mechanism by which ADP is produced. The importance of ATPases in maintaining glycolysis has been demonstrated by Meyerhof and his associates (*21, 22*). For example, in yeast extracts hexosediphosphate accumulates because of the low ATPase activity of these preparations, resulting in a lack of phosphate acceptors (ADP). Addition of ATPase brings about the formation of ADP which in turn produces a marked stimulation of fermentation (*23*). This lack of ATPase explains why glucose is fermented more rapidly by yeast extracts than is hexosediphosphate [Harden-Young effect (*23*)]: in contrast to the latter, fermentation of glucose or of fructose-6-phosphate results in the generation of ADP. In the intact yeast cell ATPase activity must be considerably greater than in cell-free extracts, because during the preparation of the latter most of the ATPases are lost by destruction or adsorption on insoluble particles (*24*). A reverse picture prevails in homogenates of brain, of tumor, and of embryonic tissues (*24*). In these preparations ATPase activity is considerably higher than is hexokinase activity; therefore, a deficiency of ATP rather than of ADP prevails. Under these conditions glycolysis is stimulated markedly by inhibitors of ATPases (decyl alcohol, digitonin, sodium azide), by adsorption of ATPase on cell particles removed by centrifugation (brain), or by the addition of hexokinase (*25–28*). Another reaction which can affect the concentration of ADP and of ATP is catalyzed by the enzyme creatine phosphokinase (*29*).

$$\text{Phosphocreatine} + \text{ADP} \leftrightarrow \text{ATP} + \text{Creatine}$$

Rapid regeneration of ATP, and thus a possible deficiency in ADP, may occur also through oxidative phosphorylations (see Section II, C).

From the foregoing, it is evident that the maintenance of a proper balance between the concentrations of ATP and of ADP must be of considerable importance in determining the rate of glycolysis in the intact cell.

C. The Pasteur Effect

In a great variety of cells, glycolysis is reduced in the presence of air. The mechanisms involved in this inhibition of carbohydrate utilization by oxygen, first described by Pasteur, have proved extremely elusive to experimental analysis (*30, 31*).

Since lactic acid, produced during glycolysis, can be resynthesized to glycogen or to glucose by the use of oxidative energy, respiration may produce an apparent decrease in carbohydrate utilization. Therefore, although aerobiosis may not decrease glycolysis necessarily, more carbohydrate would be present in the cell under aerobic than under anaerobic conditions. (32). Probably other factors play a more important role in producing the Pasteur effect.

Aerobic metabolism may result in a deficiency of ADP and (or) of inorganic phosphate. Oxidative phosphorylations generate the formation of ATP from ADP and inorganic phosphate. If the latter were in limited supply, glycolysis would be inhibited because of the required participation of inorganic phosphate in the oxidation of glyceraldehyde-3-phosphate to 2,3-diphosphoglycerate (Reaction 6). It has been pointed out above (Section II, B) that glycolysis can be inhibited because of a deficiency of ADP as a phosphate acceptor from 2,3-diphosphoglycerate (Reaction 7) and from phosphopyruvate (Reaction 10). The possibility of such a mechanism is indicated by the observations that compounds which uncouple oxidative phosphorylations (e.g., dinitrophenol) and thereby increase the availability of inorganic phosphate and of ADP, reduce or even abolish the Pasteur effect (*33–36*). Furthermore, addition of mitochondria to a reconstructed system consisting of purified enzymes and coenzymes of glycolysis results in a marked reduction of glucose uptake and of lactic acid formation. This is associated with a decrease in ADP and in inorganic phosphate (*37, 38*). Therefore, competition between mitochondrial and glycolytic enzymes for limiting amounts of ADP and of inorganic phosphate can result in an inhibition of glycolysis.

On the other hand, using brain extracts as a source of glycolytic enzymes, Aisenberg *et al.* (*39*) have reported that inhibition of glycolysis by mitochondria is not associated with a depletion of ADP or of

inorganic phosphate, but with a decrease in fructose phosphates. These investigators ascribe the Pasteur effect to an inhibition of phosphofructokinase and perhaps also of hexokinase activity, produced by a compound formed during oxidative phosphorylation. Whether or not the activity of these enzymes was inhibited under these conditions was not determined. The discrepancies in the results obtained with the combined use of soluble glycolytic enzymes and mitochondria may be due to differences in the experimental conditions, and to variations in the source and purity of glycolytic enzymes and the ratios between particulate and soluble material. Interpretation of these data is complicated further by the problem of whether, and to what extent, these experimental conditions approach those prevailing in the intact cell. On the other hand, the evidence obtained with cell-free preparations are of great value in pointing out possible mechanisms operating in the intact cell.

The possibility that the Pasteur effect may be due to a direct inhibitory effect of oxygen on glycolytic enzymes (especially SH-containing enzymes) or of oxidative metabolism has been pointed out by Lippmann (*31*) and by Engelhardt and Sakov (*40*).

While the mechanism of the Pasteur effect has not been elucidated fully, it is evident that its operation insures an efficient utilization of energy and a conservation of carbohydrate for the cell, because energy yield from the oxidation of glucose is over ten times greater than from its conversion to lactic acid.

Although the Pasteur effect has been demonstrated in numerous cells and tissues, it does not occur in all organisms. For example, utilization of glucose is the same under aerobic and anaerobic conditions in the parasitic trematodes *Schistosoma mansoni* (*41*) and *Fasciola hepatica* (*42*), and in the nematodes *Ascaris lumbricoides* (*43*), *Parascaris equorum* (*44*), *Ascaridia galli* (*45*), and *Dracunculus insignis* (*46*). These helminths take up oxygen when it is available to them, but they do not depend on respiratory metabolism for survival (*47*, *48*). Possibly these parasites do not derive or utilize energy from oxidative metabolism. If this were the case the Pasteur effect, by reducing fermentations, would deprive these organisms from a source of energy. Other helminths, such as the larvae of *Eustrongylides ignotus* (*49*) or *Litomosoides carinii* (*50*), are dependant on respiratory metabolism. In these two nematodes a Pasteur effect has been demonstrated. Therefore the operation of the Pasteur effect in a given organism might indicate that the latter is able to utilize energy generated from oxidative metabolism.

III. Glycolysis in Specialized Tissues and Cells

A. Bacteria

The occurrence of the Embden-Meyerhof scheme of phosphorylating glycolysis has been observed in numerous bacteria. However, in many of them this is not the only mechanism of carbohydrate utilization. At least two other pathways have been demonstrated.

1. *The Pentose Phosphate Pathway* (*51, 52*)

Following the dehydrogenation of glucose-6-phosphate (*53, 54*), the latter is oxidized to pentosephosphate (*53, 55–61*) and hexosephosphate is regenerated through the actions of transketolase (*51, 62–65*) and of transaldolase (*66–68*). Transketolase catalyzes the conversion of 2 moles of pentosephosphate to 1 mole of sedoheptulose-7-phosphate and 1 mole of glyceraldehyde-3-phosphate. These products yield fructose-6-phosphate and a tetrosephosphate, probably erythrose-4-phosphate (transaldolase reaction). This series of reactions also occurs in liver (*64, 69*) and in plants (*64*).

2. *The 2-Keto-3-desoxy-6-phosphogluconate Pathway*

Certain pseudomonads and aerobacters oxidize glucose to gluconic acid without prior phosphorylation (*70–75*). This is brought about by the action of a glucose oxidase (*76–77*). Two pathways of gluconate metabolism have been found in these organisms. (*a*) Gluconate is phosphorylated in the presence of ATP by gluconokinase (*78, 79*) to 6-phosphogluconate which undergoes enzymatic degradation and rearrangement to 2-keto-3-desoxy-6-phosphogluconic acid (*80–82*). This compound, through an aldolase type of reaction is degraded to glyceraldehyde-3-phosphate and to pyruvate (*80, 81, 83, 84*). Alternatively, phosphogluconate may be metabolized via the pentose phosphate route (*85, 86*) (see above). (*b*) Gluconate is oxidized to 2-ketogluconate (*74, 75, 79, 87–89*) which is phosphorylated to 2-keto-6-phosphogluconate (*79, 88*) and then reduced by TPNH to 6-phosphogluconate (*90–92*). This in turn is metabolized either via the pentose phosphate or the 2-keto-3-desoxy-6-phosphogluconate pathways.

Attempts have been made to ascertain the relative contribution of each of these pathways by the use of isotopically labeled glucose followed by the determination of the isotope distribution of various metabolic products. The limitations of this approach have been analyzed (*93*) and it is recognized that at best only semiquantitative information about this problem can be obtained in this manner (*94*). Among bac-

teria, the contribution of each of these pathways varies greatly from one species to the other. In many organisms such as in lactobacilli (*95, 96*), clostridia (*97–100*), staphylococci (*84*), streptococci (*101*), and propionic acid bacteria (*102–104*) glycolysis is the predominant, but not necessarily the exclusive pathway of carbohydrate utilization.

There is evidence for a more or less pronounced role of the pentose-phosphate pathway in many other microorganisms (*79, 86, 105–117*) and in certain pseudomonads and aerobacters the pathway via 2-keto-3-desoxy-6-phosphogluconate appears to be the major route of carbohydrate utilization (*80, 118*). Some contribution to the latter is made by the pentose phosphate route (*119*), but it is doubtful whether the Embden-Meyerhof scheme operates at all in these organisms (*120*) because cell-free extracts of pseudomonads do not ferment* glucose-6-phosphate or fructose-1,6-diphosphate, nor do they possess hexokinase or phosphofructokinase activities (*79, 80, 118*). Thus, glycolysis is by no means the exclusive mechanism of carbohydrate utilization in bacteria and the role and the contribution of the Embden-Meyerhof scheme must be ascertained in every organism whose metabolism is investigated.

B. Plants

The presence in plants of a number of glycolytic enzymes has been demonstrated (*121–124*). In the course of these studies an important difference has been uncovered between one homologous glycolytic enzyme occurring in plants and that of bacteria, yeast, and animals. The glyceraldehyde-3-phosphate dehydrogenases of the latter cells and tissues specifically react with DPN. On the other hand, three types of glyceraldehyde-3-phosphate dehydrogenases have been found in plants. One of them reacts with DPN, another one with TPN, and a third type, which is TPN-linked also, catalyzes the oxidation of glyceraldehyde-3-phosphate to 3-phosphoglycerate without the participation of phosphate (or arsenate) and without the intermediate formation of 1,3-diphosphoglycerate (*120, 125–130*). Since TPN-specific glyceraldehyde-3-phosphate dehydrogenases occur only in plant tissues exposed to light (*120, 127, 129*), it is possible that the formation of these enzymes has some relationship with photosynthesis.

Plants have been among the first tissues in which the occurrence of the pentose phosphate pathway of glucose utilization has been demonstrated (*127, 130, 131–138*) and investigations of plant enzymes have contributed significantly to an understanding of the reactions involved

* In this chapter the term *fermentation* is used for anaerobic metabolism of carbohydrate while the term *glycolysis* is reserved for the conversion of carbohydrate to lactic acid, a special case of fermentation.

in this metabolic route. Studies involving the use of glucose-1-C^{14} and of glucose-6-C^{14} have revealed that in young plant tissue carbohydrate is utilized predominantly, if not exclusively, via the Embden-Meyerhof scheme; as the plant tissues mature the contribution of the pentose phosphate pathway becomes more and more pronounced, and in older tissues up to 50% of the carbohydrate may be metabolized through this route (*135, 137, 138*).

C. Protozoa

Phototrophic and oxytrophic* protozoa utilize endogenous polysaccharides, but are unable to metabolize exogenous carbohydrate sources. These organisms accumulate large quantities of starch and use this polysaccharide as a source of energy. By contrast, glucose, mannose, and other mono- and disaccharides are not utilized by these protozoa which contain no hexokinase. It has been demonstrated (*139*) that these organisms contain a phosphorylase which catalyzes the reversible synthesis of starch from glucose-1-phosphate. Evidently, the latter cannot originate from the phosphorylation of glucose because of the absence of hexokinase, but formation of glucose-1-phosphate through photosynthetic mechanisms, or through utilization of acetate, lactate, or pyruvate, involving the formation of 2-phosphoglycerate, followed by a reversal of the Embden-Meyerhof scheme, can account for the synthesis of polysaccharide in photosynthetic and oxytrophic protozoa (*139*).

In contrast to these organisms, polytrophic or haplotrophic protozoa do not store significant amounts of polysaccharide, but utilize exogenous glucose and other carbohydrates as energy sources. Among these organisms, many trypanosomes have an exceedingly high rate of, and a marked dependence on, carbohydrate metabolism. According to calculations of Von Brand (*140*) the African pathogenic trypanosomes (*T. gambiense, T. rhodesiense, T. congolense, T. brucei, T. evansi, T. equiperdum*, and *T. hippicum*) consume in one hour an amount of glucose equivalent to 50 to 100% of their dry weight. The participation of the Embden-Meyerhof scheme in the utilization of glucose has been demonstrated in these organisms (*141–143*), although only traces of lactic acid are produced (*144, 145*) or none at all. Under anaerobic conditions 1 mole of glucose gives rise to 1 mole of glycerol and 1 mole of pyruvate while aerobically 2 moles of pyruvate are formed (*142–144, 146*). It appears that these organisms have very little lactic dehydrogenase or even lack this enzyme

* *Oxytrophic* protozoa are organisms capable of utilizing organic acids, especially lower fatty acids, as a source of energy, but unable to utilize sugars and amino acids. These organisms are distinguished from *haplotrophic* or *polytrophic* protozoa whose major energy requirements are met by the metabolism of sugars (*139*).

completely, and that under anaerobic conditions oxidation of 1 mole of glyceraldehyde-3-phosphate is coupled with the reduction of another mole of this compound to the glycerophosphate.

$$\text{Glyceraldehyde-3-phosphate} + P_i + DPN^+ \rightarrow \text{1,3-Diphosphoglycerate} + DPNH \quad (1)$$

$$\text{Glyceraldehyde-3-phosphate} + DPNH \rightarrow \text{Glycerophosphate} + DPN^+ \quad (2)$$

Hydrolysis of glycerophosphate by the action of a phosphatase would give rise to one mole of glycerol per mole of pyruvate formed from 1,3-diphosphoglycerate. If aerobically DPNH were reoxidized by an electron transport system present in these trypanosomes, reduction of glyceraldehyde-3-phosphate could not occur, resulting in the formation of two moles of pyruvate from one mole of glucose.

TABLE II
METABOLIC CHARACTERISTICS OF TRYPANOSOMES

Group	Species of *Trypanosoma*	Glucose utilization (milligrams per 100 million per hour)	Respiratory quotient	Inhibition of O_2 uptake by cyanide, 0.001 *M* (in per cent)
A	*T. equiperdum*	0.8–2.8	0.00–0.11	0
	T. evansi	0.8–2.8	0.00–0.11	0
	T. rhodesiense	0.8–2.8	0.00–0.11	0
	T. gambiense	0.8–2.8	0.00–0.11	0
	T. hippicum	0.8–2.8	0.00–0.11	0
	T. brucei	0.8–2.8	0.00–0.11	0
	T. equinum	0.8–2.8	0.00–0.11	0
B	*T. congolense*	0.53	1.0	59
C	*T. lewisi*	0.1–0.2	0.9–1.05	83–100
	T. cruzi	0.1–0.2	0.9–1.05	83–100

A high rate of carbohydrate utilization is not characteristic of all species of trypanosomes. For example, little glucose is used by *T. lewisi* and even less by *T. cruzi* (*147*, *148*). Probably, in these organisms carbohydrate is used more efficiently by a more complete oxidation of fermentation products, mediated by the transfer of electrons through the cytochrome-cytochrome oxidase, or a related heavy-metal-containing respiratory enzyme system (*144*, *149*). This is suggested also by two other metabolic differences between these two groups of trypanosomes (see Table II): (*a*) The oxygen uptake of *T. lewisi* and of *T. cruzi* is inhibited strongly by cyanide while respiration of those trypanosomes (*T. rhodesiense*, *T. gambiense*, *T. equiperdium*, *T. brucei*, *T. hippicum*, *T.*

equinum, T. evansi) which have a high rate of carbohydrate utilization is not cyanide-sensitive (*143, 144, 148, 150, 151*); (*b*) the respiratory quotient of the latter organisms is extremely low (*146, 151, 152*) while it is high in *T. cruzi* (*144, 153*) and in *T. lewisi* (*144, 146, 154, 155*), suggesting the occurrence of oxidative decarboxylations in the latter group of organisms. It is noteworthy that in these respects *T. congolense* occupies a somewhat intermediate position between these two groups (*150, 151, 156*) and that these biochemical differentiations closely parallel the morphological and taxonomic classification of trypanosomes (*157*).

Another group of protozoa in which the Embden-Meyerhof scheme is operating at a high rate are the erythrocytic stages of malarial parasites (*158*). Anaerobically, these organisms, in contrast to trypanosomes, quantitatively convert glucose to lactic acid (*159–162*). Glucose, as well as lactic acid, are oxidized completely to carbon dioxide and water; since the rate of glycolysis of malarial parasites is considerably higher than that of carbohydrate oxidation, lactic acid accumulates even under aerobic conditions; thus, a considerable amount of energy, generated aerobically, originates from glycolytic reactions. Yet, the organisms do not multiply under completely anaerobic conditions (*163*); therefore, at least some of the energy required for reproduction of malarial parasites cannot be supplied by glycolysis.

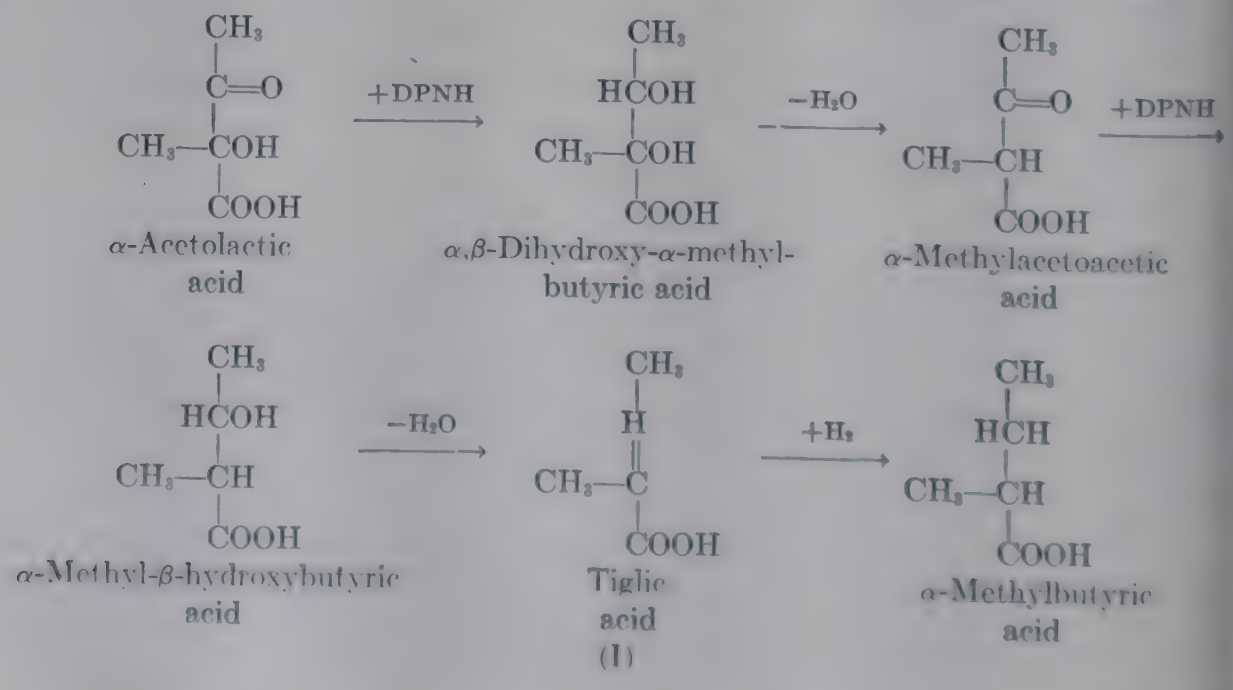
While the occurrence and the role of glycolysis has been demonstrated in many protozoa, little information is available about alternate pathways of glucose utilization in these organisms. Recently it has been reported that formation of glyceraldehyde-3-phosphate and of pyruvate from phosphogluconate and from 2-keto-3-desoxy-6-phosphogluconate probably represents the major pathway of glucose utilization of *Endamoeba histolytica* (*164*). Therefore, the existence of this pathway is not restricted to a relatively limited number of bacteria (pseudomonads, aerobacters).

D. Helminths

A common characteristic of these invertebrate metazoa is a high rate of carbohydrate metabolism, associated with incomplete substrate oxidation. This not only applies to intestinal helminths whose habitat is nearly anaerobic, but also to parasitic worms, such as schistosomes, which live in oxygen-rich surroundings. Although helminths take up oxygen when it is available to them, fermentation rates remain high, even under aerobic conditions. Many of these organisms depend for survival on anaerobic metabolism and appear to be unable to utilize energy derived from oxidative reactions (*145, 165*).

A variety of fermentation products are formed by helminths. Many of them produce lactic acid, but only rarely is the latter the exclusive or even the major end product of carbohydrate metabolism, as in the case of *Schistosoma mansoni* (*41*) and *Dracunculus insignis* (*46*). Volatile fatty acids [acetic (*50, 166*), propionic (*166, 167*), *n*-valeric (*168, 169*), α-methylbutyric (*167*), tiglic (*167*) acid, and one or several unidentified C_6-volatile acids (*167, 169*)], higher nonvolatile fatty acids (*170–172*), succinic acid (*170, 171, 173*) and acetylmethylcarbinol (*174, 175*) have been identified as products of the carbohydrate metabolism of parasitic worms. In every species in which this problem has been studied, it has been found that the Embden-Meyerhof scheme represents the major pathway for the utilization of carbohydrate by helminths (*167, 176–181*). The occurrence of the pentose-phosphate route has been demonstrated in *Ascaris* (*182, 183*), but the reported low activities of the enzymes involved in this pathway suggest that quantitatively the latter plays only a minor role in the utilization of carbohydrate by *Ascaris*. It has not been determined whether phosphogluconate can be metabolized in helminths via 2-desoxy-3-keto-6-phosphogluconate.

No definite information is available about the mechanisms involved in the formation of volatile acids or of succinic acid by helminths. It is conceivable that pyruvate, produced by glycolytic reactions, is the common precursor of these acids. Recently it has been demonstrated that α-acetolactate is formed from pyruvate by *Ascaris* muscle (*175*). As illustrated below, the carbon skeleton of α-acetolactate is the same as that of α-methylbutyric and tiglic acids which are fermentation products of *Ascaris*. Saz *et al.* (*175*) have pointed out that these acids could be formed from α-acetolactate by the sequence (I) of reactions shown.



(I)

This hypothesis is supported by the finding that *Ascaris* muscle contains enzyme systems which catalyze the reduction of α-acetolactate and of α-methylacetoacetate by DPNH (*175*). The activities of these reducing systems are considerably higher than that of lactic dehydrogenase of *Ascaris* muscle (*184*). The formation of considerable quantities of succinate by *Ascaris* (*173*) and by other helminths (*170, 171*) also could originate with pyruvate and may proceed through CO_2 fixation, catalyzed by the "malic" enzyme, followed by a conversion of malate to succinate via fumarate. Such a reaction sequence would be consistent with the high "malic" enzyme (*185*), fumarase (*185*), and succinic dehydrogenase (*186, 187*) activities of *Ascaris* muscle.

An increased energy requirement produces a shift from fatty acid to lactic acid fermentation in the liver fluke *Fasciola hepatica*. The major products of carbohydrate metabolism of this helminth are propionate and acetate (in an approximate ratio of 3:1) while only 4 to 8% of the utilized carbohydrate is converted to lactate (*166*). Stimulation of the muscular activity of the parasite by low concentration of 5-hydroxytryptamine or of lysergic acid diethylamide results in a two- to sixfold increase in lactate production while there is little or no change in propionate or acetate formation (*166, 188*). It would appear that in these organisms lactic acid fermentation can meet increased energy requirements more efficiently than fatty acid fermentation.

E. Insects

An interesting variant of glycolysis has been described recently in the extremely active flight muscle of the housefly (*Musca domestica*) (*189*) and of some Hymenoptera. In these species, lactic dehydrogenase activity is of a very low order when compared with crayfish or rat muscle; on the other hand, the activity of α-glycerophosphate dehydrogenase is very high (*190, 191*). All the other glycolytic enzymes concerned with the production of pyruvic acid from hexoses are present in these insect flight muscles (*192, 193*) as well as enzymes of the pentose phosphate pathway (*194, 195*) and of the tricarboxylic acid cycle (*193, 196*). Unlike the muscle of higher animals, lactic acid is oxidized slowly, if at all, in fly flight muscle preparations (*193*). Glycerophosphate has been found to accumulate in insect muscle (*197*).

On the basis of these and other findings, including the discovery of a very active non-pyridine-linked glycerophosphate dehydrogenase in flight muscle mitochondria (*190, 193, 198–201*), Sacktor has suggested that glycogen, glucose, and several other sugars are metabolized to pyruvic acid via the Embden-Meyerhof scheme present in the sarcoplasm or nonparticulate part of the muscle (*189*). However, DPNH, produced

during triosephosphate oxidation, is not reoxidized by means of the reduction of pyruvate to lactate catalyzed by lactic dehydrogenase; this reoxidation is brought about by the reduction of dihydroxyacetone phosphate to glycerophosphate. Glycerophosphate in turn is rapidly oxidized to dihydroxyacetone phosphate by a non-pyridine-linked dehydrogenase present in the sarcosomes (mitochondria). The electron transfer from glycerophosphate to oxygen occurs through the cytochrome system with the generation of ATP with a P : O ratio approaching 2 (*200*). The sarcosomal oxidation of glycerophosphate is at least 10 times as active as the oxidation of any of the substrates of the Krebs tricarboxylic acid cycle and apparently is sufficiently large to account for the very high increase in oxygen uptake which occurs when the muscle passes from the resting to the active state (*189, 201*).

F. Vertebrates

1. *Normal Tissues*

Glycolysis is of widespread occurrence in the tissues of mammals and of other vertebrates. Incubation of surviving preparations of virtually every organ or tissue studied results in the formation of lactic acid from added glucose or from endogenous glycogen (*30, 202–208*). However, the rate of glycolysis is very variable both under aerobic and anaerobic conditions. Slices of some tissues, such as liver or kidney cortex, produce relatively small amounts of lactic acid even anaerobically, whereas preparations of other cells, such as retina, jejunal mucosa, and kidney medulla, are much more active both in the presence or absence of oxygen. All tissues exhibit a marked Pasteur effect.

Poorly understood, however, is the importance of lactic acid production from glucose or glycogen in the economy of the cells of higher animals. Since the majority of organs or tissues are unable to withstand severe anoxia for more than minutes, it is obvious that *anaerobic* glycolysis cannot sustain the life of such cells except in a few instances (see below).

Aerobically, the evaluation of the possible importance of lactic acid production from carbohydrate is complicated because the same Embden-Meyerhof glycolytic scheme is used for the production of pyruvate, and because of the occurrence in animal tissues of an alternate mechanism for the initial breakdown of carbohydrate, i.e., the pentose phosphate pathway (*51, 64, 69*). Pyruvate production via glycolysis is considered to be of great importance in the preparation of hexoses and possibly other sugars for the subsequent generation of most of the energy in the carbohydrate molecule. However, even here, the *quantitative* importance

of the Embden-Meyerhof pathway as yet is unknown in most tissues because of the almost ubiquitous occurrence of the alternate pentose phosphate shunt. The problem of interpreting comparative results of the initial oxidation of various labeled glucose and other hexose molecules has been one of the barriers to final elucidation of this important problem of cellular metabolism (*93, 94*).

Another aspect of glycolysis which also needs clarification is its possible importance in those metabolic activities of the cell not involved directly with energy production. For example, it is considered probable that the degradation of glucose-6-phosphate by the pentose phosphate pathway is important in the formation of reduced TPN for some synthetic reactions (*209–213a*). Does the generation of reduced DPN by the oxidative step in the glycolytic scheme have any importance in cell metabolism other than in mitochondrial oxidative phosphorylation? Does the apparent intracellular localization of the enzymes of both glycolysis (*214*) and the hexose phosphate pathway (*215*) in the nonparticulate portions of the cell have any special functional significance in providing both reduced pyridine nucleotide coenzymes for synthesis and for other special activities of cell particulates?

Despite the uncertainties concerned with the metabolic role of glycolysis in most animal tissues, there are a few types of cells in which the position of glycolysis is somewhat better understood. Skeletal muscle probably is the best example (*216*). This is one of the few tissues of higher animals capable of incurring a significant oxygen debt. In muscle, glycolysis is capable of generating sufficient ATP for replenishment of the energy stores. However, even here, glycolysis by itself is only effective for relatively short periods of time.

Another interesting example of the role of glycolysis in cell physiology is found in mammalian spermatozoa (*217*). In many species, these cells show a variant of the classical glycolysis in that the predominant primary substrate for glycolysis is fructose, rather than glucose or glycogen (*218, 219*). Apparently, spermatozoan motility and function can be maintained in large measure by the glycolytic degradation of fructose, called fructolysis, by the Embden-Meyerhof pathway from fructose-6-phosphate to lactic acid. Although glucose is metabolized as easily as is fructose, the physiological substrate appears to be fructose. In the testis and epidydymis, where apparently fructose is not available, the sperm are immobile. However, they become motile on their passage down to the urethra, where fructose is added to the seminal fluid from the accessory organs of reproduction, such as the seminal vesicles. Spermatozoa in common with most other tissues, exhibit a Pasteur effect. Mammalian erythrocytes also appear to use glycolysis as an important

metabolic pathway, since apparently the integrity of these cells is in some way dependent on glycolysis (*220*).

Glycolysis is also very active in blood leucocytes (*221–224*), but still undefined is the exact relationship of this metabolic pathway to the cell integrity of different leucocytes, and to their many functions. In most instances, the observation of high rates of glycolysis in special anatomic sites, such as jejunal mucosa, retina, and renal medulla remains interesting but as yet unintegrated into our knowledge of cell behavior. Recent findings with some tissues in which glycolysis is quantitatively greater than is respiration, such as in cornea or in lens tissue, have demonstrated the pronounced dependence of the cell integrity on the respiratory activity even though the latter is very low (*225, 226*).

It has been known for a long time that the rate of glycolysis is very high in cells and organs of the developing embryo and that the level of glycolysis shows interesting variations at different stages of embryologic growth and differentiation (*207, 227*). However, despite the relatively large amount of experimentation in this field, the possible role of glycolysis in determining and modifying over-all cell function and behavior is not understood.

2. *Neoplastic Tissues*

One of the most intriguing facts related to the problem of the comparative biochemistry of glycolysis is that malignant tumors, with very rare exceptions (*221, 222*), exhibit much higher rates of aerobic and anaerobic glycolysis than do most normal homologous adult tissues. Since this was reported by Warburg and co-workers over 30 years ago (*201*), it has been confirmed repeatedly in a wide variety of animal and human malignant tumors (*30, 203–206, 208*). In fact, a high rate of aerobic glycolysis can be considered as one of the few metabolic aberrations which have been found consistently to occur in cancer cells.

As with most normal tissues, the rate of glycolysis in tumors is higher anaerobically than aerobically, i.e., most tumors show a Pasteur effect. Whether the magnitude of this effect is smaller in tumors than in normal tissues is difficult to state, since it depends upon the manner of expression. Absolutely, many neoplastic tissues show decreases in the rate of glycolysis in the presence of oxygen as large or larger than do most normal tissue. However, since both anaerobic and aerobic glycolysis rates are elevated, on a percentage basis, the effect usually is considerably less in the neoplastic tissue. Many malignant tumors also show another interesting relationship between glycolysis and respiration, namely the Crabtree effect (*228*). Increasing glucose concentrations in the medium produce a progressive decrease in the rate of respiration of

the surviving cells. So far this effect has been reported only rarely (*223, 224, 229*) in normal tissues.

The basis for the increased rate of glycolysis in tumors is still not established. The following are some of the possible factors that have been considered: (*a*) a different mechanism of glycolysis in tumors than in normal tissues; (*b*) different properties, such as rates or substrate-enzyme affinities, in the enzymes of the two kinds of tissues; (*c*) increased amounts of the rate-limiting enzymes and other enzymes of glycolysis; (*d*) alterations in the intracellular regulation of the metabolic pathways; or (*e*) alterations in the permeability of cancer cells.

a. Qualitatively, it is generally accepted that the enzymatic mechanisms of glycolysis in tumors are the same as those in normal tissues (*230–234*), despite the occasional claims for "nonphosphorylating glycolysis" and other alternate schemes for the Embden-Meyerhof pathway (*235, 236*).

b. In the very few instances where homologous enzymes of tumors and of normal tissues have been studied, the enzymes from malignant tissues were found to be indistinguishable immunologically from those of normal tissues of the same species (*237, 238*).

c. Studies of the activities of glycolysis or of one or more of the glycolytic enzymes in cell-free preparations have yielded confusing results. For example, LePage has found that homogenates of many normal rat tissues produced as much as or more lactic acid than did homogenates of several malignant rat tumors when incubated anaerobically in the presence of hexosediphosphate, glucose, pyruvate, and fluoride (*234*). Also, lactic dehydrogenase activity of several malignant tissues was in the same range as that of normal tissues (*206*). In contrast, some studies have demonstrated considerably higher activities for many of the glycolytic enzymes of malignant tissues as compared to homologous normal tissues (*239–241*). Also, a high-malignancy tumor line grown in tissue culture was reported to have considerably more activity with regard to some glycolytic enzymes than did a more normal line of cells (*242*). The resolution of this apparent discrepancy between the results of several investigators will be of considerable importance for the eventual interpretation of the significance of the high rate of glycolysis in cancer tissue.

d. Several recent studies have been concerned with clarifying the possible role of intracellular factors, such as the level of inorganic phosphate and the levels of ATP and ADP, upon the balance between respiration and glycolysis in the intact cells [(*27, 28, 243–248*); see also Section II above]. These investigators indicate a probable critical role of the concentrations of inorganic phosphate and/or of phosphate ac-

ceptors in the regulation of respiration and glycolysis and in the mechanism of the Pasteur and Crabtree effects in tumor cells. However, they have not as yet pinpointed the essential basis for the high rates of glycolysis *per se* in malignant neoplastic cells.

e. The high rate of glucose uptake by cancer cells (*249*) has been suggested as a possible basis for the high rate of glycolysis in tumors (*250*). The implication that the high rates of glycolysis in tumor cells may be due to the high rate of glucose phosphorylation at low concentrations of glucose (*251*) is compatible with this hypothesis.

The relative importance of glycolysis as compared with respiration in the generation of energy for the needs of tumor cells has yet to be defined in a quantitative manner. Because of the high rate of glycolysis of cancer cells, the proportion of ATP produced by glycolysis is theoretically large in tumors (*242*). However, the actual energy requirements of cancer cells and the relative amount of this energy generated by glycolysis is still unknown. Since cancer cells in general appear to have fewer alternate metabolic pathways for many substrates (*252*), it is possible that their energy requirements differ radically from those of normal cells, qualitatively as well as quantitatively. This factor would have to be considered in any evaluation of the relative rates of glycolysis and respiration in energy production.

IV. Species and Tissue Specificities of Glycolytic Enzymes

One of the most fundamental properties of enzymes is their specificity for a substrate or for a group of substrates. In addition, along with other proteins, they are endowed with species specificities. Enzymes which are found in different species are not necessarily identical with each other, although they catalyze the same reactions, and although they may have the same substrate specificities. The species specificity of enzymes is of obvious interest to comparative biochemistry and has been demonstrated in several studies concerned with properties of homologous glycolytic enzymes.

The first demonstration of the existence of such species differences among glycolytic enzymes was supplied by Krebs and Najjar (*252*). Following immunization of rabbits and chickens with crystalline glyceraldehyde-3-phosphate dehydrogenase of yeast, the serums of these animals inhibited the activity of this enzyme, but had no effect on glyceraldehyde-3-phosphate dehydrogenase of rabbit muscle. Similarly, an antiserum produced against yeast hexokinase had an inhibitory effect on this enzyme, but did not affect the catalytic activity of brain hexokinase (*253*). As a result of repeated injections of lactic dehydrogenase of rabbit muscle into roosters, an antiserum was obtained which markedly

reduced the activity of this enzyme; its inhibitory potency on lactic dehydrogenase of rat muscle was significantly lower while it had no effect on the lactic dehydrogenase of schistosomes (*254*). Conversely, following immunization of roosters against lactic dehydrogenase of *Schistosoma mansoni*, the serums of these animals decreased the activity of schistosome lactic dehydrogenase, but not that of lactic dehydrogenase of rabbit muscle (*255*).

Incubation of phosphoglucose isomerase of schistosomes with an antiserum against this enzyme produced a sharp decline in the activity of the schistosome isomerase, but did not interfere with the catalytic function of this enzyme in the same reaction in rabbit muscle (*180*). The antiserum against the schistosome isomerase did not affect the activity of lactic dehydrogenase of the same parasite and, conversely, the immune serum against the latter enzyme had no effect on the isomerase of schistosomes (*256*). Because of the absence of cross-reactions, it is evident that these antiserums react with sites specific for a particular enzyme, rather than with groupings common to schistosome proteins.

The specificity of enzyme-antibody reactions is illustrated also by the observations that the antiserum against yeast glyceraldehyde-3-phosphate dehydrogenase had no effect on yeast hexokinase (*252*), and that the antiserum against rabbit muscle lactic dehydrogenase did not affect the activities of phosphoglucose isomerase, of aldolase, and of glyceraldehyde-3-phosphate dehydrogenase of rabbit muscle (*254*), indicating again that these antibodies react with sites specific for a particular enzyme of a given species. While the species specificity of enzymes has been tested only in a limited number of instances, it should be pointed out that there has been no case where homologous enzymes from different species were found to be identical. A recent investigation of Henion and Sutherland (*256*) has revealed that an enzyme may be specific not only for a particular species, but also for a single tissue or organ of the same species; an antiserum to dog heart phosphorylase inhibited this enzyme but had no effect on dog liver phosphorylase. Preincubation of the enzyme with the substrate or coenzyme afforded protection from the inhibitory effects of some of the antiserums (*180, 255*). It appears that, in these instances, at least one point of attachment of the antibody is localized either at the active center of the enzyme or in such close proximity to the active center that combination of the substrate or coenzyme with the enzyme prevented interaction with the antibody.

Unless the active center is involved in a more direct manner, the protective effects of coenzymes do not prove necessarily that the active centers, rather than some other constituents of homologous enzymes, differ from each other, particularly if the functional characteristics of

these enzymes are identical. This applies to the phosphoglucose isomerases of *Schistosoma mansoni* and of rabbit muscle (*180*). The dissociation constants of the substrates are identical, as are the equilibrium constants of both enzymes, and there are close similarities in the pH optima. Thus, the two enzymes can be distinguished from each other only by specific antibodies. On the other hand, when differences in kinetics or in cofactor requirements of homologous enzymes are observed, the question may be raised whether or not their active centers are identical. Kinetic differences have been reported in the cases of lactic dehydrogenases (*257*) and of the phosphofructokinases (*181*) of schistosomes and rabbit muscle.

The aldolases of mammalian muscle (*258, 259*), of *Trypanosoma cruzi* (*260*), and of plants (*261, 262*) have no metal requirements, while the activities of the aldolases of yeast (*258*), of *Clostridium perfringens* (*263*), of *Aspergillus niger* (*264*), and of trichomonads (*265, 266*) are inhibited by metal-binding compounds, such as α,α-dipyridyl. This inhibition is reversed by Fe^{2+}. While the latter is the only divalent metal activating the clostridial aldolase (*263*), the enzymes catalyzing the same reaction in yeast and in *Aspergillus* are activated also by Zn^{2+}, Co^{2+}, and Mn^{2+} (*258, 264*). An even greater degree of difference in the nature of the active center must prevail when variations in the substrate specificity are encountered among homologous enzymes. Such is the case with the hexokinases of various species and tissues. Some of these enzymes [yeast (*267, 268*), brain (*267*)] catalyze the phosphorylation of glucose, fructose, mannose, glucosamine, and desoxyglucose. On the other hand, numerous hexokinases from other tissues and organisms have a high specificity for a single hexose (*179, 269–281*).

Differences in the nature of homologous enzymes of various species should be reflected in variations in susceptibility of these enzymes to certain inhibitors. This is illustrated not only by the effect of metal-binding agents on aldolases (see above), but also by the actions of organic antimonials on phosphofructokinases (*282*). Low concentrations of these compounds markedly inhibit the activity of the schistosome phosphofructokinase, while interference with the catalytic activity of the host's phosphofructokinase occurs only with much higher concentrations or does not occur at all. This differential susceptibility of the phosphofructokinase of the parasite to antimonials can account for the chemotherapeutic action of the latter in schistosomiasis. Glycolysis supplies the major source of energy for the organism and the rate of the phosphofructokinase reaction determines the rate of glycolysis of the parasite. Inhibition of phosphofructokinase activity of schistosomes by antimonials is demonstrable also in the intact parasite because admin-

istration of subcurative doses of stibophen to the host results in an accumulation of the substrate (fructose-6-phosphate) and a decrease of the product (fructose-1,6-diphosphate) of the phosphofructokinase reaction within the parasite (*181*).

While the specificity of homologous glycolytic enzymes for various species and tissues has been demonstrated, there is no evidence that glycolytic enzymes of muscle and of tumors of the same animal differ from each other. Kubowitz and Ott (*237*) have found that immunologically, as well as kinetically, the lactic dehydrogenases of rat muscle and of Jensen rat sarcoma are indistinguishable from each other. Similar results have been obtained with aldolase of rabbit muscle and of rabbit Brown-Pearce tumor cells (*238*).

From the foregoing, it is evident that differences exist in the nature of glycolytic enzymes which catalyze the same reactions in different species and tissues. Such differences may occur at various levels. In some cases, homologous glycolytic enzymes may be distinguished only by the use of specific antibodies. In other instances, such enzymes differ from each other not only immunologically but also in their affinities for their substrates, in the effect of the hydrogen ion concentration on their activities, and in their cofactor requirements. In other cases, differences in substrate specificities as well as in kinetics are encountered. Finally, certain compounds interfere with the functional integrity of a glycolytic enzyme of one species, but not with that of another. Such differences are pertinent to an understanding of the mechanism of action and to the rational development of chemotherapeutic agents; it remains to be determined whether and in what manner such variations can be related to biochemical evolution and adaptation.

References

1. H. A. Krebs and H. L. Kornberg, *Ergeb. Physiol.* **49,** 212–298 (1957).
2. G. Gomori, *J. Biol. Chem.* **148,** 139–149 (1943).
3. H. G. Hers, *Biochim. et Biophys. Acta* **8,** 416–423 (1952).
4. L. C. Mokrasch and R. W. McGilvery, *J. Biol. Chem.* **221,** 909–917 (1956).
5. G. T. Cori, C. F. Cori, and G. Schmidt, *J. Biol. Chem.* **129,** 629–639 (1939).
6. M. A. Swanson, *J. Biol. Chem.* **184,** 647–659 (1950).
7. R. Levine, M. S. Goldstein, B. Huddlestun, and S. P. Klein, *Am. J. Physiol.* **163,** 70–76 (1950).
8. M. S. Goldstein, V. Mullick, B. Huddlestun, and R. Levine, *Am. J. Physiol.* **173,** 207–216 (1953).
9. C. R. Park, R. L. Post, C. F. Kalman, J. H. Wright, Jr., L. H. Johnson, and H. E. Morgan, *Ciba Fdn. Colloquia Endocrin.* **9,** 240–260 (1956).
10. C. R. Park, J. Bornstein, and R. L. Post, *Am. J. Physiol.* **182,** 12–16 (1955).
11. C. R. Park and L. H. Johnson, *Am. J. Physiol.* **182,** 17–23 (1955).
12. C. R. Park, L. H. Johnson, and J. H. Wright, Jr., *Federation Proc.* **15,** 324 (1956).

13. E. J. Huycke and P. Kruhoffer, *Acta Physiol. Scand.* **34,** 232–249 (1955).
14. R. B. Fisher and D. B. Lindsay, *J. Physiol.* **131,** 526–541 (1956).
15. H. I. Nakada, T. N. Morita, and A. N. Wick, *J. Biol. Chem.* **115,** 803–808 (1955).
16. H. I. Nakada, *J. Biol. Chem.* **219,** 319–326 (1956).
17. J. Bornstein and C. R. Park, *J. Biol. Chem.* **205,** 503–511 (1953).
18. H. E. Morgan and C. R. Park, *J. Clin. Invest.* **36,** 916 (1957).
19. E. Bueding and C. Swartzwelder, *Pharmacol. Revs.* **9,** 329–365 (1957).
20. C. R. Park, Personal communications, (1958).
21. O. Meyerhof, *J. Biol. Chem.* **157,** 105–119 (1945).
22. O. Meyerhof and J. R. Wilson, *Arch. Biochem.* **23,** 246–255 (1949).
23. A. Harden and W. J. Young, *Proc. Roy. Soc.* **B80,** 299–311, (1908).
24. O. Meyerhof, *Wallerstein Labs. Commun.* **12,** 255–265 (1949).
25. O. Meyerhof and J. R. Wilson, *Arch. Biochem.* **17,** 153–169 (1948).
26. O. Meyerhof and N. Geliazkowa, *Arch. Biochem.* **12,** 405–434 (1947).
27. O. Meyerhof and J. R. Wilson, *Arch. Biochem.* **21,** 22–34 (1949).
28. O. Meyerhof and J. R. Wilson, *Arch. Biochem.* **21,** 1–21 (1949).
29. O. Meyerhof and J. R. Wilson, *Arch. Biochem.* **14,** 71–82 (1947).
30. D. Burk, *Cold Spring Harbor Symposia Quant. Biol.* **7,** 420–459 (1939).
31. F. Lippmann, *in* "A Symposium on Respiratory Enzymes," pp. 48–71. University of Wisconsin Press, Madison, Wisconsin, 1942.
32. O. Meyerhof, *Biochem. Z.* **162,** 43–86 (1925).
33. E. C. Dodds and G. D. Greville, *Lancet* **i,** 398–399 (1934).
34. O. Meyerhof and S. Fiala, *Biochim. et Biophys. Acta* **6,** 1–12 (1951).
35. E. H. Newcomb, *Am. J. Botany* **37,** 264–279 (1950).
36. H. Beevers, *Am. J. Botany* **40,** 91–95 (1953).
37. S. Gatt, I. Krimsky, and E. Racker, *Federation Proc.* **15,** 259–260 (1956).
38. S. Gatt, *Federation Proc.* **16,** 184–185 (1957); S. Gatt and E. Racker, *J. Biol. Chem.* **234,** 1024–1028 (1959).
39. A. C. Aisenberg, B. Reinafarje, and V. R. Potter, *J. Biol. Chem.* **224,** 1099–1113 (1957); A. C. Aisenberg and V. R. Potter, *ibid.* pp. 1115–1127; A. C. Aisenberg, *ibid.* **234,** 441–444 (1959).
40. V. A. Engelhardt and N. E. Sakov, *Biokhimiya* **8,** 9 (1943); *Chem. Abstr.* **37,** 6680 (1943).
41. E. Bueding, *J. Gen. Physiol.* **33,** 475–495 (1950).
42. T. E. Mansour, unpublished observations (1958).
43. T. von Brand, *Z. vergleich. Physiol.* **21,** 220–235 (1934).
44. Y. Toryu, *Sci. Repts. Tôhoku Imp. Univ., Fourth Ser.* **10,** 687–696 (1936).
45. W. M. Reid, *J. Parasitol.* **31,** 406–410 (1945).
46. E. Bueding and J. Oliver-Gonzales, *Brit. J. Pharmacol.* **5,** 62–64 (1950).
47. E. Bueding and L. Peters, *J. Pharmacol. Exptl. Therap.* **101,** 210–229 (1951).
48. E. Bueding, L. Peters, S. Koletsky, and D. V. Moore, *Brit. J. Pharmacol.* **8,** 15–18 (1953).
49. T. von Brand and W. F. Simpson, *Proc. Soc. Exptl. Biol. Med.* **60,** 368–371 (1945).
50. E. Bueding, *J. Exptl. Med.* **89,** 107–130 (1949).
51. E. Racker, *Advances in Enzymol.* **15,** 141–182 (1954).
52. I. C. Gunsalus, B. L. Horecker, and W. A. Wood, *Bacteriol. Revs.* **19,** 79–128 (1955).

53. O. Warburg, W. Christian, and A. Griese, *Biochem. Z.* **282,** 157–205 (1935).
54. D. B. M. Scott and S. S. Cohen, *Biochem. J.* **55,** 23–33 (1953).
55. O. Warburg and W. Christian, *Biochem. Z.* **292,** 287–295 (1937).
56. F. Dickens, *Biochem. J.* **32,** 1645–1653 (1938).
57. D. B. M. Scott and S. S. Cohen, *J. Biol. Chem.* **188,** 509–530 (1951).
58. B. Horecker, P. Z. Smyrniotis, and J. E. Seegmiller, *J. Biol. Chem.* **193,** 383–396 (1951).
59. P. Srere, J. Cooper, V. Klybas, and E. Racker, *Arch. Biochem. Biophys.* **59,** 535 (1955).
60. J. Hurwitz and B. L. Horecker, *J. Biol. Chem.* **223,** 993–1008 (1956).
61. B. L. Horecker, P. Z. Smyrniotis, and J. Hurwitz, *J. Biol. Chem.* **223,** 1009–1018 (1956).
62. B. L. Horecker and P. Z. Smyrniotis, *J. Am. Chem. Soc.* **74,** 2123 (1952).
63. B. L. Horecker and P. Z. Smyrniotis, *J. Am. Chem. Soc.* **75,** 1009–1010 (1953).
64. B. L. Horecker, P. Z. Smyrniotis, and H. Klenow, *J. Biol. Chem.* **205,** 661–682 (1953).
65. G. de la Haba, I. G. Leder, and E. Racker, *J. Biol. Chem.* **214,** 409–426 (1955).
66. B. L. Horecker and P. Z. Smyrniotis, *J. Biol. Chem.* **212,** 811–825 (1955).
67. B. L. Horecker, P. Z. Smyrniotis, H. Hiatt, and P. Marks, *J. Biol. Chem.* **212,** 827–836 (1955).
68. M. Gibbs and B. L. Horecker, *J. Biol. Chem.* **208,** 813–820 (1954).
69. B. L. Horecker, M. Gibbs, H. Klenow, and P. Z. Smyrniotis, *J. Biol. Chem.* **207,** 393–403 (1954).
70. K. R. Butlin, *Biochem. J.* **30,** 1870–1871 (1936).
71. L. B. Lockwood, B. Tabenkin, and G. E. Ward, *J. Bacteriol.* **42,** 51–61 (1941).
72. F. N. Stokes and J. J. R. Campbell, *Arch. Biochem.* **30,** 121–125 (1951).
73. C. A. Claridge and C. H. Werkman, *Arch. Biochem. Biophys.* **47,** 99–106 (1953).
74. H. Katznelson, *J. Bacteriol.* **70,** 469–475 (1955).
75. J. H. Dowling and H. B. Levine, *J. Bacteriol.* **72,** 555–560 (1956).
76. H. K. Schachman, A. B. Pardee and R. Y. Stanier, *Arch. Biochem. Biophys.* **38,** 245–260 (1952).
77. W. A. Wood and R. F. Schwerdt, *J. Biol. Chem.* **201,** 501–511 (1953).
78. S. S. Cohen, *J. Biol. Chem.* **189,** 617–628 (1951).
79. S. A. Narrod and W. A. Wood, *J. Biol. Chem.* **220,** 45–55 (1956).
80. W. A. Wood and R. F. Schwerdt, *J. Biol. Chem.* **206,** 625–635 (1954).
81. J. MacGee and M. Doudoroff, *J. Biol. Chem.* **210,** 617–626 (1954).
82. R. Kovachevich and W. A. Wood, *J. Biol. Chem.* **213,** 745–756 (1955).
83. N. Entner and M. Doudoroff, *J. Biol. Chem.* **196,** 853–862 (1952).
84. L. S. Fosdick and G. W. Rapp, *Arch. Biochem.* **1,** 379–389 (1943).
85. J. T. Sokatch, A. P. Prieto and I. C. Gunsalus, *Bacteriol. Proc.* p. 112 (1956).
86. P. J. Vandemark and W. A. Wood, *J. Bacteriol.* **71,** 385–392 (1956).
87. A. Dalby and A. C. Blackwood, *Can. J. Microbiol.* **1,** 733–742, (1955).
88. J. DeLey, *Biochim. et Biophys. Acta* **13,** 302 (1954).
89. T. Ramakrishnan and J. S. R. Campbell, *Biochim. et Biophys. Acta* **17,** 124–127 (1955).

90. J. DeLey and S. Verhofstede, *Enzymologia* **18,** 47–62 (1957).
91. E. W. Frampton and W. A. Wood, *Bacteriol. Proc.* p. 122 (1957).
92. A. C. Blackwood and E. R. Blankley, *Bacteriol. Proc.* pp. 121–122 (1957).
93. H. G. Wood, *Physiol. Revs.* **35,** 841–859 (1955).
94. M. F. Utter, *Ann. Rev. Biochem.* **27,** 245–284 (1958).
95. M. Gibbs, R. Dumross, F. A. Bennett, and M. R. Bubeck, *J. Biol. Chem.* **184,** 545–549 (1950).
96. L. Pine, V. Haas, and H. A. Barker, *J. Bacteriol.* **68,** 227–230 (1954).
97. H. A. Barker and M. D. Kamen, *Proc. Natl. Acad. Sci. U. S.* **31,** 219–225 (1945).
98. H. G. Wood, *J. Biol. Chem.* **199,** 579–583 (1955).
99. L. M. Page, M. Gibbs, and R. C. Bard, *J. Bacteriol.* **72,** 65–67 (1956).
100. R. Gavard, *Compt. rend.* **238,** 1620–1622 (1954).
101. O. J. O'Kane and W. W. Umbreit, *J. Biol. Chem.* **142,** 25–30 (1942).
102. H. G. Wood, R. W. Stone, and C. H. Werkman, *Biochem. J.* **31,** 349–359 (1937).
103. W. P. Wiggert and C. H. Werkman, *Biochem. J.* **32,** 101–107 (1938).
104. H. G. Wood, R. G. Kulka, and N. L. Edson, *Biochem. J.* **62,** 177–182 (1956).
105. D. B. McNair Scott and S. S. Cohen, *J. Biol. Chem.* **188,** 509–530 (1951).
106. S. S. Cohen, *Bacteriol Revs.* **15,** 131–146 (1951); *Science* **123,** 653–656 (1956).
107. P. A. Kitos, T. E. King, and V. H. Cheldelin, *Federation Proc.* **15,** 289–290 (1956).
108. A. H. Bokman, M. R. Lusby, J. H. Dowling, and H. B. Levine, *Bacteriol. Proc.* p. 117 (1956).
109. M. Damodaran, V. Jagannathan, and K. Singh, *Enzymologia* **17,** 199–206 (1955).
110. W. A. Volk and D. N. Myrvik, *Am. Rev. Tuberc. Pulmonary Diseases* **73,** 196–201 (1956).
111. M. Gibbs, J. M. Earl, and J. L. Ritchie, *J. Biol. Chem.* **217,** 161–168 (1955).
112. P. J. Vandemark and G. M. Fukui, *J. Bacteriol.* **72,** 610–614 (1956).
113. J. E. Zajic, J. DeLey, and M. P. Starr, *Bacteriol. Proc.* p. 113, (1956).
114. E. M. Jensen, H. Altschuller, and R. C. Bard, *Bacteriol. Proc.* p. 113, (1956).
115. J. G. Hauge, T. E. King, and V. H. Cheldelin, *J. Biol. Chem.* **214,** 1–9, 11–26 (1955).
116. L. E. Mortenson and P. Wilson, *J. Biol. Chem.* **213,** 713–721 (1955).
117. E. D. Bergmann, U. Z. Littauer, and B. E. Volcani, *Biochim. et Biophys. Acta* **13,** 288–289 (1954).
118. W. A. Wood, *Bacteriol. Revs.* **19,** 222–233 (1955).
119. J. DeLey, *Enzymologia* **18,** 33–46 (1957).
120. D. I. Arnon, L. L. Rosenberg, and F. R. Whatley, *Nature* **173,** 1132–1133 (1954).
121. P. K. Stumpf, *Ann. Rev. Plant Physiol.* **3,** 17–34 (1952).
122. P. K. Stumpf, *in* "Phosphorus Metabolism" (W. D. McElroy and B. Glass, eds.), Vol. 2, pp. 29–67, Johns Hopkins Press, Baltimore, Maryland, 1952.
123. A. Millerd, J. Bonner, B. Axelrod, and R. S. Bandurski, *Proc. Natl. Acad. Sci. U. S.* **37,** 855–862 (1951).
124. P. D. Saltman, *J. Biol. Chem.* **200,** 145–154 (1953).
125. P. K. Stumpf, *J. Biol. Chem.* **182,** 261–272 (1950).

126. S. Tewfik and P. K. Stumpf, *J. Biol. Chem.* **192,** 519–533 (1951).
127. M. Gibbs, *Nature* **170,** 164–165 (1952).
128. D. I. Arnon, *Science* **116,** 635–637 (1952).
129. R. H. Hagerman and D. I. Arnon, *Arch. Biochem. Biophys.* **55,** 162–168 (1955); **57,** 421–426 (1955).
130. B. Axelrod, R. S. Bandurski, C. M. Greiner, and R. Jang, *J. Biol. Chem.* **202,** 619–634 (1953).
131. E. E. Conn and B. Vennesland, *J. Biol. Chem.* **192,** 17–28 (1951).
132. B. Vennesland and E. E. Conn, *Ann. Rev. Plant Physiol.* **3,** 307–332 (1952).
133. B. Axelrod, R. S. Bandurski, C. M. Greiner, and R. Jang, *J. Biol. Chem.* **202,** 619–634 (1954).
134. R. C. Barnett, H. A. Stafford, E. C. Conn, and B. Vennesland, *Plant Physiol.* **28,** 115–122 (1953).
135. H. Beevers and M. Gibbs, *Plant Physiol.* **29,** 318–321, 322–324 (1954).
136. M. Gibbs, *Plant Physiol.* **29,** 34–39 (1954).
137. M. Gibbs and H. Beevers, *Plant Physiol.* **30,** 343–347 (1957).
138. B. Axelrod and H. Beevers, *Ann. Rev. Plant Physiol.* **7,** 267–298 (1956).
139. A. Lwoff, ed., "Biochemistry and Physiology of Protozoa," Vol. I, 1–26. Academic Press, New York, 1951.
140. T. von Brand, *in* "Biochemistry and Physiology of Protozoa" (A. Lwoff, ed.), Vol. I, 177–234. Academic Press, New York, 1951.
141. G. Chen and E. M. K. Geiling, *Proc. Soc. Exptl. Biol. Med.* **63,** 486–487 (1946).
142. P. B. Marshall, *Brit. J. Pharmacol.* **3,** 8–14 (1948).
143. S. C. Harvey, *J. Biol. Chem.* **179,** 435–453, (1949).
144. J. F. Ryley, *Biochem. J.* **62,** 215–222 (1956).
145. T. von Brand, "Chemical Physiology of Endoparasitic Animals." Academic Press, New York, 1952.
146. L. Reiner, C. V. Smythe, and J. T. Pedlow, *J. Biol. Chem.* **113,** 75–88 (1936).
147. T. von Brand, *Z. vergleich. Physiol.* **19,** 587–614 (1933).
148. T. von Brand and E. J. Tobie, *J. Cellular Comp. Physiol.* **31,** 49–68 (1948).
149. H. D. Baernstein and E. J. Tobie, *Federation Proc.* **10,** 159 (1951).
150. T. von Brand and E. M. Johnson, *J. Cellular Comp. Physiol.* **29,** 33–50 (1947).
151. T. von Brand, E. J. Tobie, and B. Mehlman, *J. Cellular Comp. Physiol.* **35,** 273–300 (1950).
152. S. R. Christophers and J. D. Fulton, *Ann. Trop. Med. Parasitol.* **82,** 43–75 (1938).
153. T. von Brand, E. M. Johnson, and C. W. Rees, *J. Gen. Physiol.* **30,** 163–175 (1946).
154. J. W. Moulder, *J. Infectious Diseases* **83,** 42–49 (1948).
155. J. F. Riley, *Biochem. J.* **49,** 577–585 (1951).
156. M. Agosin and T. von Brand, *Exptl. Parasitol.* **3,** 517–524 (1954).
157. C. A. Hoare, *Proc. 4th Intern. Congr. Trop. Med., Washington, D. C.,* (1948) **2,** 1110–1116 (1948).
158. J. F. Speck and E. A. Evans, *J. Biol. Chem.* **159,** 71–81 (1945).
159. W. B. Wendel, *J. Biol. Chem.* **148,** 21–34 (1943).
160. M. Silverman, J. Ceithaml, L. G. Taliaferro, and E. A. Evans, *J. Infectious Diseases* **75,** 212–230 (1944).

161. J. F. Speck, J. W. Moulder, and E. A. Evans, *J. Biol. Chem.* **164,** 119–114 (1946).
162. R. W. McKee, R. A. Ormsbee, C. B. Anfinsen, Q. M. Geiman, and E. G. Ball, *J. Exptl. Med.* **84,** 569–582 (1946).
163. R. W. McKee, *in* "Biochemistry and Physiology of Protozoa" (A. Lwoff, ed.), Vol. I, pp. 251–322. Academic Press, New York, 1951.
164. D. M. Hilker and A. G. C. White, *Federation Proc.* **17,** 242 (1958).
165. E. Bueding, *Physiol. Revs.* **29,** 195–218 (1949).
166. T. E. Mansour, *Federation Proc.* **15,** 454 (1956).
167. E. Bueding and H. W. Yale, *J. Biol. Chem.* **193,** 411–423 (1951).
168. E. Bueding, *J. Biol. Chem.* **202,** 505–512 (1953).
169. T. von Brand, P. P. Weinstein, B. Mehlman, and E. C. Weinbach, *Exptl. Parasitol.* **1,** 245–255 (1952).
170. H. L. Alt and O. A. Tischer, *Proc. Soc. Exptl. Biol. Med.* **29,** 222–224 (1931).
171. T. von Brand, *Z. vergleich. Physiol.* **18,** 562–596 (1933).
172. E. Weinland and T. von Brand, *Z. vergleich. Physiol.* **4,** 212–285 (1926).
173. E. Bueding and G. W. Farrow, *Exptl. Parasitol.* **5,** 345–349 (1956).
174. S. Berl and E. Bueding, *J. Biol. Chem.* **191,** 401–418 (1951).
175. H. J. Saz, A. Vidrine, Jr., and J. A. Hubbard, *Exptl. Parasitol.* **7,** 477–490 (1958).
176. W. P. Rogers and M. Lazarus, *Parasitology* **39,** 302–314 (1949).
177. C. P. Read, *Exptl. Parasitol.* **1,** 1–18 (1951).
178. L. Rathbone and K. R. Rees, *Biochim. et Biophys. Acta* **15,** 126–133 (1954).
179. E. Bueding and J. A. MacKinnon, *J. Biol. Chem.* **215,** 495–506 (1955).
180. E. Bueding and J. A. MacKinnon, *J. Biol. Chem.* **215,** 507–513 (1955).
181. E. Bueding and J. M. Mansour, *Brit. J. Pharmacol.* **12,** 159–165 (1957).
182. J. DeLey and R. Vercruysee, *Biochem. et Biophys. Acta* **16,** 615–616 (1955).
183. N. Entner, *Arch. Biochem. Biophys.* **71,** 52–61 (1957).
184. E. Bueding, unpublished observations (1956).
185. H. J. Saz and J. A. Hubbard, *J. Biol. Chem.* **225,** 921–933 (1957).
186. E. Bueding and B. Charms, *J. Biol. Chem.* **196,** 615–627 (1952).
187. E. Bueding, N. Entner, and E. Farber, *Biochim. et Biophys. Acta* **18,** 305–306 (1955).
188. T. E. Mansour and A. D. Lago, *J. Pharmacol. Exptl. Therap.* **122,** 748A (1958).
189. B. Sacktor, *in* "Biochemistry of Insects" (L. Levenbook, ed.), pp. 150–152. Pergamon, New York.
190. E. C. Zebe and W. H. McShan, *J. Gen. Physiol.* **40,** 779–790 (1957).
191. B. Sacktor and D. G. Cochran, *Biochim. et Biophys. Acta* **25,** 649 (1957).
192. W. Chefurka, *Enzymologia* **17,** 73–89 (1954).
193. B. Sacktor, *J. Biophys. Biochem. Cytol.* **1,** 29–46 (1955).
194. W. Chefurka, *Enzymologia* **18,** 209–227 (1957).
195. W. Chefurka, *Can. J. Biochem. Physiol.* **36,** 83–102 (1958).
196. D. D. Hoskins, V. H. Cheldelin, and R. W. Newburgh, *J. Gen. Physiol.* **39,** 705–713 (1956).
197. V. Kubista, *Nature* **180,** 549 (1957).
198. B. Sacktor and D. Cochran, *J. Am. Chem. Soc.* **78,** 3227 (1956).
199. E. C. Zebe, *Experientia* **12,** 68–70 (1956).
200. B. Sacktor and D. Cochran, *Arch. Biochem. Biophys.* **74,** 266–276 (1958).

201. B. Chance and B. Sacktor, *Arch. Biochem. Biophys.* **76,** 509–531, (1958).
202. O. Warburg, "Stoffwechsel der Tumoren," Springer, Berlin, 1926, English transl., "The Metabolism of Tumours," by F. Dickens, Constable, London, 1930.
203. F. Dickens, *Cancer Rev.* **6,** 57–74 (1931).
203a. F. Dickens, *in* "The Enzymes" (J. B. Sumner and K. Myrbäck, eds.), Vol. II, Pt. 1, pp. 624–683. Academic Press, New York, 1951.
204. D. Burk, *Am. Assoc. Advancem. Sci. Occasional Publs.* **4,** 121–161 (1937).
205. E. Boyland, *in* "Die Methoden der Fermentforschung" (E. Bamann and K. Myrbäck, eds.). Thieme, Leipzig, 1941; reprint, pp. 2240–2265, Academic Press, New York, 1945.
206. J. P. Greenstein, "Biochemistry of Cancer," 2nd ed. Academic Press, New York, 1954.
207. J. Needham, "Chemical Embryology," Cambridge University Press, London and New York, 1931.
208. S. Weinhouse, *Advances in Cancer Research* **3,** 269–325 (1955).
209. C. de Duve and H. G. Hers, *Ann. Rev. Biochem.* **26,** 149–180 (1957).
210. R. G. Langdon, *J. Am. Chem. Soc.* **77,** 5190–5192 (1955).
211. H. A. Krebs, *Bull. Johns Hopkins Hosp.* **95,** 19–33 (1954).
212. M. F. Utter and K. Kurahashi, *J. Biol. Chem.* **207,** 821–841 (1954).
213. S. Kit and A. C. Griffin, *Cancer Research* **18,** 621–656 (1958).
213a. M. D. Siperstein and V. M. Fagan, *J. Clin. Invest.* **37,** 1185–1195, 1196–1201 (1958).
214. G. A. LePage and W. C. Schneider, *J. Biol. Chem.* **176,** 1021–1027, (1948).
215. R. W. Newburgh and V. H. Cheldelin, *J. Biol. Chem.* **218,** 89–95, (1956).
216. M. Dubuisson, "Muscular Contraction." C. C Thomas, Springfield, Illinois, 1954.
217. T. Mann, *Advances in Enzymol.* **9,** 329–390 (1949).
218. T. Mann, *Biochem. J.* **40,** 481–491 (1946).
219. T. Mann, *Nature* **157,** 79 (1946).
220. O. F. Denstedt, *in* "Blood Cells and Plasma Proteins" (J. L. Tullis, ed.), pp. 223–253. Academic Press, New York, 1953.
221. W. S. Beck and W. N. Valentine, *Cancer Research* **12,** 818–822 (1952).
222. W. S. Beck and W. N. Valentine, *Cancer Research* **12,** 823–828 (1952).
223. G. R. McKinney, S. P. Martin, R. W. Rundles, and R. Green, *J. Appl. Physiol.* **5,** 335–340 (1935).
224. S. P. Martin, G. R. McKinney, and R. Green, *Ann. N. Y. Acad. Sci.* **59,** 996–1002 (1955).
225. A. Pirie and R. Van Heyningen, "The Biochemistry of the Eye," Blackwell Sci. Publs., Oxford University Press, London and New York, 1956.
226. J. E. Harris, J. D. Hauschildt, and L. T. Nordquist, *Am. J. Ophthalmol.* **38,** 148–151 (1954).
227. J. Brachet, "Chemical Embryology," 2nd ed. Interscience, New York, 1950.
228. H. G. Crabtree, *Biochem. J.* **23,** 536–545 (1929).
229. L. H. Cohen, *Federation Proc.* **16,** 713 (1957).
230. E. Boyland and M. E. Boyland, *Biochem. J.* **29,** 1910–1914 (1935).
231. E. Boyland, M. E. Boyland, and G. D. Greville, *Biochem. J.* **31,** 461–466 (1937).
232. G. A. LePage, *J. Biol. Chem.* **176,** 1009–1020 (1948).

233. A. B. Novikoff, V. R. Potter and G. A. LePage, *Cancer Research* **8**, 203–210 (1948).
234. G. A. LePage, *Cancer Research* **8**, 197–200 (1948).
235. D. P. Barr, E. Ronzoni, and J. Glaser, *J. Biol. Chem.* **80**, 331–344, (1928).
236. F. H. Scharles, M. D. Baker, and W. T. Salter, *Biochem. J.* **29**, 1927–1936 (1935).
237. F. Kubowitz and P. Ott, *Biochem. Z.* **314**, 94–117 (1943).
238. P. LaBorde, unpublished data.
239. M. Villavicencio and E. S. G. Barron, *Arch. Biochem. Biophys.* **67**, 121–137 (1957).
240. G. Weber and A. Cantero, *Cancer Research* **17**, 995–1005 (1957).
241. J. A. Sibley and G. A. Fleischer, *Cancer Research* **15**, 609–611 (1955).
242. O. Warburg, *Science* **123**, 309–314 (1956).
243. E. Racker, *Ann. N. Y. Acad. Sci.* **63**, 1017–1021 (1956); R. Wu and E. Racker, *J. Biol. Chem.* **234**, 1029–1035, 1036–1041 (1959).
244. B. Chance and B. Hess, *Ann. N. Y. Acad. Sci.* **63**, 1008–1016 (1956).
245. E. Kvamme, *Acta. Physiol. Scand.* **42**, 204–218 (1958).
246. E. Kvamme, *Acta Physiol. Scand.* **42**, 239–250 (1958).
247. G. Acs and F. B. Straub, *Doklady Akad. Nauk S.S.S.R.* **95**, 1021–1024 (1954); *Chem. Abstr.* **48**, 9525 (1954).
248. M. Brin and R. W. McKee, *Cancer Research* **16**, 364–368 (1956).
249. R. K. Crane, R. A. Field, and C. F. Cori, *J. Biol. Chem.* **224**, 649–662 (1957).
250. L. Bloch-Frankenthal and S. Weinhouse, *Cancer Research* **17**, 1082–1090 (1957).
251. V. R. Potter, *Federation Proc.* **17**, 691–697 (1958).
252. E. G. Krebs and V. A. Najjar, *J. Exptl. Med.* **88**, 569–577 (1948).
253. R. E. Miller, V. Z. Posternak, and G. M. Sevag, *J. Bacteriol.* **58**, 621–625 (1949).
254. T. E. Mansour, E. Bueding, and A. B. Stavitsky, *Brit. J. Pharmacol.* **9**, 182–186 (1954).
255. W. H. Henion, T. E. Mansour, and E. Bueding, *Exptl. Parasitol.* **4**, 40–44 (1955).
256. W. F. Henion and E. W. Sutherland, *J. Biol. Chem.* **224**, 477–488 (1957).
257. T. E. Mansour and E. Bueding, *Brit. J. Pharmacol.* **8**, 431–434 (1953).
258. O. Warburg and W. Christian, *Biochem. Z.* **314**, 149–176 (1943).
259. J. F. Taylor, A. A. Green, and G. T. Cori, *J. Biol. Chem.* **173**, 591–604 (1948).
260. H. D. Baernstein and C. W. Rees, *Exptl. Parasitol.* **1**, 215–228 (1952).
261. P. K. Stumpf, *J. Biol. Chem.* **176**, 233–241 (1948).
262. S. Tewfik, and P. K. Stumpf, *Am. J. Botany* **36**, 567–577 (1949).
263. R. C. Bard and I. C. Gunsalus, *J. Bacteriol.* **59**, 387–400 (1950).
264. V. Jagannathan, K. Singh, and M. Damodaran, *Biochem. J.* **63**, 94–105 (1956).
265. H. D. Baernstein, *Federation Proc.* **13**, 177 (1954).
266. J. F. Ryley, *Biochem. J.* **59**, 361–369 (1955).
267. L. Berger, M. W. Slein, S. P. Colowick, and C. F. Cori, *J. Gen. Physiol.* **29**, 379–391 (1946).
268. M. Kunitz and M. R. McDonald, *J. Gen. Physiol.* **29**, 393–412 (1946).
269. R. Santo, S. Takemori, and M. Ebata, *J. Biochem. (Tokyo)* **43**, 623–633 (1956).

270. R. E. Trucco, R. Caputto, L. F. Leloir, and N. Mittelman, *Arch. Biochem.* **18,** 137–146 (1948).
271. M. W. Slein, G. T. Cori, and C. F. Cori, *J. Biol. Chem.* **186,** 763–780 (1950).
272. H. P. Klein and M. Doudoroff, *J. Bacteriol.* **59,** 739–750 (1950).
273. C. E. Cardini, *Enzymologia* **14,** 362–368 (1951).
274. G. T. Cori, S. Ochoa, M. W. Slein, and C. F. Cori, *Biochim. et Biophys. Acta* **7,** 304–317 (1951).
275. F. Leuthardt and E. Testa, *Helv. Chim. Acta* **34,** 931–938 (1951).
276. E. Bueding, H. Ruppender, and J. MacKinnon, *Proc. Natl. Acad. Sci. U. S.* **40,** 773–777 (1954).
277. A. Sols, *Biochim. et Biophys. Acta* **20,** 62–68 (1956).
278. A. Medina and A. Sols, *Biochim. et Biophys. Acta* **19,** 378–379 (1956).
279. M. Doudoroff, W. J. Palleroni, J. MacGee, and M. Ohara, *J. Bacteriol.* **71,** 196–201 (1956).
280. N. J. Palleroni, R. Contopoulou, and M. Doudoroff, *J. Bacteriol.* **71,** 202–207 (1956).
281. H. W. Kosterlitz, *Biochem. J.* **31,** 2217–2224 (1937).
282. T. E. Mansour and E. Bueding, *Brit. J. Pharmacol.* **9,** 459–462 (1954).

CHAPTER 10

Comparative Biochemistry of Electron Transport and Oxidative Phosphorylation

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I. Introduction

In their recent review, Krebs and Kornberg (*1*) divide the release of energy from foodstuffs by living organisms into three phases. The first of these phases is composed of processes in which the foodstuffs are converted to smaller component molecules. The carbohydrates are converted to hexoses, proteins to amino acids, and fats to glycerol and fatty acids. Relatively little energy is released in these reactions and that which is, appears as heat.

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In Phase II the hexoses, fatty acids, glycerol, and amino acids are converted to one of three compounds, acetyl-CoA, α-ketoglutarate, or oxalacetate. In Phase III these three intermediates in turn are oxidized to CO_2 and H_2O through the reactions of the tricarboxylic acid (Krebs) cycle. In contrast to Phase I, the energy released in Phases II and III is relatively large, and much of this energy is made available to the organism.

Most of the energy available in foodstuffs is released when hydrogen atoms are removed from oxidizable substrates, in turn reacting with O_2 to form water. The over-all reaction is represented in highly simplified form by Eq. 1.

$$\text{Substrate} + \tfrac{1}{2}\,O_2 \rightarrow \text{Oxidized Substrate} + H_2O \qquad (1)$$

The free energy changes (ΔF°) for oxidations encountered in Phases II and III range from —35 to —70 kcal. per mole of substrate (*1*). In many of the oxidation reactions, the immediate oxidizing agent is one of the pyridine nucleotides,* DPN^+ or TPN^+ (Eq. 2).

$$\text{Substrate} + \text{Oxidized pyridine nucleotide} \rightarrow \text{Oxidized substrate} + \text{Reduced pyridine nucleotide} \qquad (2)$$

Thus in the glycolytic sequence reduced pyridine nucleotides are formed when glyceraldehyde-3-phosphate, lactate, and pyruvate are oxidized. The oxidation of glycerol and thioesters of β-hydroxy fatty acids leads to DPNH formation, and DPNH or TPNH is produced as a result of the oxidation of malate, isocitrate, and α-ketoglutarate in the Krebs cycle. The oxidative deamination of glutamic acid yields DPNH or TPNH.

In some instances the oxidizing agent is a flavoprotein instead of a pyridine nucleotide (Eq. 3).

$$\text{Substrate} + \text{Oxidized flavoprotein} \rightarrow \text{Oxidized substrate} + \text{Reduced flavoprotein} \qquad (3)$$

The oxidation of succinate, the thioesters of fatty acids and the oxidative deamination of amino acids are examples of reactions in which flavoproteins participate.

The free energy changes in reactions of the type shown in Eqs. 2 and 3 are usually not large; they may range from —10 to +10 kcal. These values follow from a comparison of the oxidation-reduction potentials of the various substrates with those for the pyridine nucleotides and flavoproteins. Instead the large release of energy described in Eq. 1 occurs when the reduced coenzymes are reoxidized by molecular O_2.

* Abbreviations: DPN^+ and DPNH, TPN^+ and TPNH, oxidized and reduced forms of di- and triphosphopyridine nucleotide; GSSG and GSH, oxidized and reduced glutathione; FAD, flavin adenine dinucleotide; AA, ascorbic acid; DHA, dehydroascorbic acid; AMP, ADP, and ATP, adenosine mono-, di-, and triphosphate; P_i, inorganic phosphate; DNP, dinitrophenol; CoA, coenzyme A.

For example, the standard free energy change at pH 7.0 for the oxidation of DPNH by O_2 is −52 kcal./mole (Eq. 4).

$$DPNH + H^+ + \tfrac{1}{2} O_2 \rightarrow DPN^+ + H_2O \quad (4)$$

The several ways in which the reduced carriers can be oxidized by O_2 will be discussed. However, of greatest significance to the organism are those pathways of oxidation in which free energy can be conserved and made available for its use. When this consideration is taken into account, predominant in interest is the electron transport pathway from reduced nucleotide to O_2 which involves flavin, the cytochromes, and cytochrome oxidase. It is now well established that energy is conserved by the formation of energy-rich phosphate bonds as electron transfer occurs from reduced pyridine nucleotide to oxygen through the cytochrome chain. The comparative aspects of this process, which is termed oxidative phosphorylation, will be discussed. Energy is also conserved in the formation of acyl phosphate and thioester bonds during the oxi dation of glyceraldehyde-3-phosphate and the α-keto acids pyruvate and α-ketoglutarate. The mechanisms of these reactions are fairly well understood but will not be discussed here, since the comparative biochemistry of glycolysis (2) and the Krebs cycle (3) are discussed elsewhere in this treatise.

At this point it is well to re-emphasize, as have Krebs and Kornberg, the simplication inherent in the processes just described. Through these processes, a large number of different organic compounds are first degraded to simpler units (hexose, amino acids, and fatty acids) and then converted to three organic acids. Partly during this latter stage and during the oxidation of the organic acids, a limited variety of reduced carrier compounds (DPNH, TPNH, and reduced flavoprotein) are formed. Finally, during the oxidation of these reduced carriers, free energy is released and made available to the organism in the form of energy-rich phosphate compounds. The means by which this last is accomplished is now discussed in some detail.

II. The Cytochrome Respiratory Chain

A. Components of the Chain

1. *Cytochromes*

From the historical standpoint the first components of the cytochrome respiratory chain to be described were the cytochromes themselves, discovered and named "histohematins" by MacMunn (4) in 1886. In 1925 Keilin (5) confirmed MacMunn's discovery and showed that at least three cellular pigments, "cytochromes," were widely dis-

tributed in living cells. The only exception appeared to be certain anaerobic bacteria (*6*).

The most obvious property of the cytochromes was their ability to absorb light in characteristic regions of the spectrum. The pigments were defined in terms of position of the absorption maxima. In heart muscle cytochromes a, b, and c absorbed maximally at 605, 562, and 550 mμ respectively. The absorption of the cytochromes not only permitted identification of the pigments but also provided clues to their chemical nature and to their biological role.

In 1933 Keilin (*6*) claimed an important role for the cytochromes in cellular respiration based on observations of the following nature. The cytochromes were almost ubiquitous in nature. They were capable of alternate oxidation and reduction, a property which could be observed directly in living cells (bacteria, yeast, insects). The oxidation and reduction of the cytochromes was affected by well known inhibitors of cellular respiration such as KCN, NaN_3, and CO. One of the pigments, cytochrome c, was readily extracted from bakers' yeast and partially purified. When reduced chemically and added to a homogenate of heart muscle, the reduced cytochrome c was oxidized by O_2. The oxidized cytochrome c on the other hand was rapidly reduced when a normal cellular metabolite such as succinate was added to the muscle preparation. These last two observations clearly indicated the ability of the cytochromes to function as respiratory carriers and meant that the cytochromes were to play another important role. This was the resolution of a controversy of this period concerning biological oxidations. The cytochromes provided a functioning link between the hydrogen activation theory of Wieland and the oxygen activation concept championed by Warburg.

Warburg's contribution to the mechanism of cellular respiration resulted from his fundamental studies on the role of heavy metals as catalysts in oxidation reactions (*7*). His experiments on the inhibition of cellular respiration by various substances led him to suggest the existence of an iron enzyme linking molecular oxygen to cell metabolism. Warburg named this enzyme *Atmungsferment* and provided evidence that it was a heme-protein. His studies on the photodissociation of the CO-inhibited enzyme led to the publication of the relative absorption spectrum of this enzyme in 1929 (*8*). An enzyme system, indophenol oxidase, which catalyzed the oxidation of a mixture of α-naphthol and paraphenylenediamine was shown by Keilin to be widely distributed in nature and was inhibited by KCN, NaN_3, and CO (the latter was photoreversible). Since this oxidase system also catalyzed the oxidation of reduced cytochrome c, Keilin renamed the enzyme cytochrome oxi-

dase (*9*). Later studies by Keilin and Hartree (*10*) revealed that the light absorption bands assigned to cytochrome a were composed of two components, cytochrome a, previously described, and cytochrome a_3. The letters a_1 and a_2 had been assigned to two cytochromes of the a type which were observed in bacteria (*11*). Cytochrome a_3, which reacted with CO in the ferrous state and was easily autoxidized, was considered to be identical with cytochrome oxidase.

Many other cytochromes of the a, b, and c types have since been described, but until recently most of these have resisted purification. This is due to their association with the insoluble component of the cell. With the discovery of numerous cytochromes there has developed a great need for a more systematic nomenclature, but little progress has been made in this direction.

It is not proposed to discuss the cytochromes themselves in more detail. Authoritative reviews (*12–16*) are available and the comparative aspects of these substances as a group are treated elsewhere in this treatise (*17*).

2. *Flavoproteins*

In 1932 the first flavoprotein, the "old yellow enzyme," was discovered by Warburg and Christian (*18*). Their experiments on the role of this enzyme in the aerobic oxidation of glucose-6-PO_4 led in turn to studies on glucose-6-phosphate dehydrogenase and the discovery of TPN^+ (*19*). Because its prosthetic group could be alternately reduced and oxidized, the old yellow enzyme was postulated to function as a carrier between TPNH and O_2. Although the reduced enzyme was oxidized by O_2 in air, it could also be oxidized by cytochrome c at an equal rate (*20*). This was the first indication that a flavoprotein might serve as a link between the pyridine nucleotides and the cytochromes. However, the rate of oxidation of the reduced old yellow enzyme by either O_2 or cytochrome was not large enough to be of physiological significance.

In 1940 Haas *et al.* (*21*) described the isolation of a soluble cytochrome reductase which catalyzed the reduction of cytochrome c preferentially by TPNH. This enzyme reacted 10^5 times as rapidly with cytochrome c as did the old yellow enzyme. In recent years cytochrome reductases have been purified from several tissues including liver microsomes (*22–24*). The latter enzyme is interesting in that it catalyzes the reduction of a microsomal cytochrome, but not cytochrome c. The soluble cytochrome reductases contain a flavin nucleotide and may contain a metal ion in addition (*25*). They can utilize various dyes and cytochrome c as acceptors, but do not react with oxygen. The reduction

of cytochrome c catalyzed by particulate preparations is more complex and is discussed in detail below (Section II, C).

Another enzymatic activity, the reduction of dyes such as methylene blue by reduced pyridine nucleotides, has been ascribed to a group of enzymes known as the diaphorases. In contrast to the cytochrome reductases, the diaphorases do not react readily with cytochrome c and consequently their physiological function, if any, is unknown. A current view holds that the diaphorases are a modified form of cytochrome reductase which no longer can react with cytochrome c (*25*).

In addition to accepting hydrogens from the reduced pyridine nucleotide, flavoproteins can react directly with metabolites. Glucose, xanthine, aldehydes, and amino acids are examples of organic substrates which can be oxidized directly by flavoproteins. The properties of these enzymes and of other flavoproteins are described in recent reviews (*25, 26*).

3. *Pyridine Nucleotides*

The pyridine nucleotide TPN^+ was discovered in 1931 (*27*) by Warburg and Christian. The observation that nicotinamide was a component of the coenzyme stimulated experiments on the mechanism of action of the nucleotide. As a result of these studies, DPN^+, discovered 30 years earlier (*28*), was shown to be closely related to TPN^+. The structure of TPN^+ remained unsettled until 1953, when the position of the third phosphate group could definitely be assigned to the 2′-hydroxyl of the ribose attached to adenine. Knowledge of the chemistry and function of the pyridine nucleotides and their dehydrogenases has progressed steadily (*29, 30*). Again the most important single property of these coenzymes is their ability to undergo reversible oxido-reductions. In the presence of the appropriate dehydrogenase, hydrogen atoms are transferred between substrate and coenzyme. The process involves a direct transfer of one of the hydrogen atoms from the substrate to the pyridine nucleotide in a stereochemically specific manner (*31*). The other hydrogen is released in solution as H^+ ion (Eq. 5).

$$\text{Substrate-}H_2 + DPN^+ \rightleftharpoons \text{Substrate} + DPNH + H^+ \qquad (5)$$

The list of dehydrogenases requiring DPN^+ or TPN^+ is large (*29, 30*). Since DPNH and TPNH can be reoxidized in the presence of oxidized substrates and the appropriate dehydrogenase, the pyridine nucleotides can serve as hydrogen carriers between two substrate molecules. The most familiar example of this action is the coupling of the oxidation of glyceraldehyde-3-phosphate to the reduction of pyruvate or acetaldehyde, which occurs during glycolysis or alcoholic fermentation. In the oxidation of cellular metabolites by molecular O_2, however, the function

of the pyridine nucleotides is to serve as the first step in the pathway of hydrogen and electron transport from substrate to oxygen.

B. Oxidation-Reduction Potentials

Further understanding of the nature of the cytochrome respiratory chain developed when oxidation-reduction potentials of some of the components were first determined. The values for the old yellow enzyme (32) ($E_0 = -0.06$ volt, pH 7.0) and cytochrome c (33) ($E_0 = +0.27$ volt) placed these carriers intermediate in value between those of most of the organic substrates (metabolites) and that of O_2 ($E_0 = +0.810$ volt, pH 7.0). Moreover, the value for DPN^+ (34) ($E_0 = -0.28$, pH 7.0) was appropriate for a stepwise transfer of electrons or hydrogen from DPN^+ to the cytochromes (Scheme I).

$$\text{Substrate} \rightarrow \begin{matrix}\text{DPN}^+ \\ \text{TPN}^+\end{matrix} \rightarrow \text{Flavoprotein} \rightarrow \text{Cytochromes} \rightarrow O_2 \qquad \text{(I)}$$

The electron transfer sequence assigned to the cytochromes is based on the oxidation-reduction potentials for cytochrome c, b, and a determined by Ball (35) in 1938. These values (E_0; pH 7.0) were -0.04 volt for cytochrome b, $+0.27$ volt for cytochrome c, and $+0.29$ volt for cytochrome a. Thus the cytochromes in Scheme I can be elaborated to give Scheme II.

$$\text{Cytochrome b} \rightarrow \text{Cytochrome c} \rightarrow \text{Cytochrome a} \rightarrow O_2 \qquad \text{(II)}$$

Keilin and Hartree's (36) observations on the complexity of the absorption bands attributed to cytochrome a resulted in further expansion to the sequence shown in Scheme III.

$$\text{Cytochrome b} \rightarrow \text{Cytochrome c} \rightarrow \text{Cytochrome a} \rightarrow \text{Cytochrome } a_3 \qquad \text{(III)}$$

The values of the oxidation-reduction potentials for the different components of the cytochrome respiratory chain were re-examined whenever better methods or purified samples of the carriers became available. Some of the values recently published are listed in Table I.* It is interesting to note that the sequence postulated 20 years ago (37) from values available at that time is the same which would be indicated by the most recent values.

C. Cellular Localization of the Respiratory Chain

1. *Non-phosphorylative Particles*

a. The Keilin and Hartree Heart Muscle Preparation. (1) Oxidation of Succinate. In 1940 Keilin and Hartree (36) described a cell-free tissue preparation from heart muscle which has been extensively used in

* See p. 459.

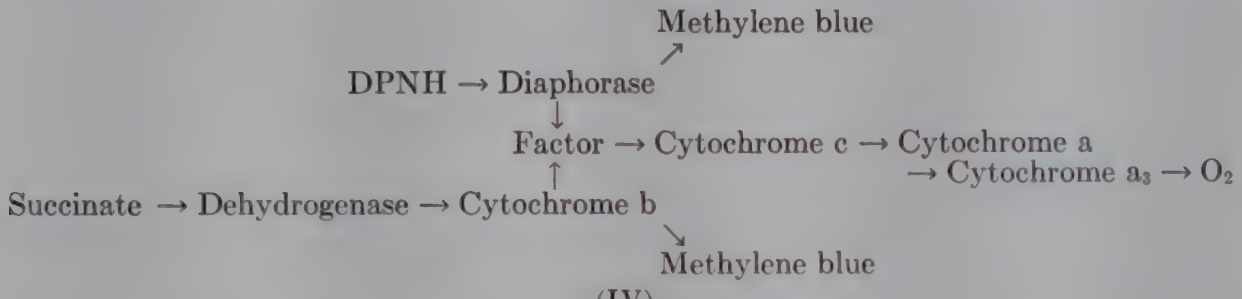
studies on the cytochrome respiratory chain. The Keilin and Hartree preparation is an insoluble preparation which catalyzes the oxidation of succinate by molecular O_2 (succinoxidase activity) through the cytochrome system. It therefore contains all of the enzymes and factors necessary to function between succinate and O_2 (cytochromes b, c, a, a_3 and additional factors). Although succinate oxidation proceeds in the absence of cytochrome c, the reaction is stimulated by the addition of cytochrome c also. The preparation will also catalyze the oxidation of ferrocytochrome c by molecular O_2 (cytochrome oxidase activity), as well as the oxidation of succinate by various acceptors such as cytochromes, ferricyanide, and different dyes. Although the latter activity is frequently referred to as succinic dehydrogenase (*38*), recent studies on the nature of the enzymes catalyzing these reactions has required further development of terminology. Singer *et al.* (*39*) use the term "succinic dehydrogenase" to mean the protein which catalyzes the reversible oxido-reduction of succinate to fumarate. This enzyme, which has been extensively purified and is an iron flavoprotein, reacts most rapidly with phenazine dyes, less rapidly with ferricyanide, and only slowly with indophenol dyes, methylene blue, or cytochrome c. Green (*40*) and co-workers use the term "succinic dehydrogenase complex" to refer to a particulate preparation which catalyzes the oxidation of succinate by cytochrome c. It must therefore contain the succinic dehydrogenase described by Singer plus additional factors capable of reacting with cytochrome c.

Our knowledge of the succinoxidase system, although extensive, is certainly not complete. This fact was stressed by Wainio and Cooperstein (*14*) who emphasized that "as study of the succinoxidase system has proceeded, more and more factors have been postulated." These authors suggest that, although such factors may exist, the existence of these components may "also be explained in terms of structural alteration" of the particulate preparations which can catalyze succinate oxidation.

(2) Oxidation of Reduced Pyridine Nucleotides. In 1950 Slater (*41*) examined the ability of the Keilin and Hartree succinoxidase preparation to oxidize DPNH. The preparation catalyzes both the oxidation of DPNH by O_2 (DPNH-oxidase activity) and that of DPNH by cytochrome c (DPNH-cytochrome c reductase activity). In his studies Slater made extensive use of inhibitors such as cyanide, carbon monoxide, and British anti-Lewisite (BAL or 2,3-dimercapto-1-propanol), which react with carriers in the cytochrome chain. Cyanide, carbon monoxide, azide, and fluoride are inhibitors which bind iron-porphyrins and can react readily with cytochrome oxidase. On the other

hand, BAL (*41, 42*), the antibiotic antimycin A (*43*), Amytal (*44*), and certain quinones (*45*) interfere with electron transfer at points between cytochrome c and succinate or DPNH. Although the mode of action of this latter group of inhibitors is not at all clear (*46*), the site of action has been specified (*47*). However, since there is not complete agreement on the sequence of carriers in the region where these inhibitors are believed to function, the site of action of the inhibitors may not be known with absolute certainty.

From his studies, Slater concluded that the respiratory chain from DPNH and succinate to O_2 had the structure shown in Scheme IV (*41, 42*).



(IV)

The heart tissue preparation was considered to contain a factor ("Slater's factor") which catalyzed the reaction between a diaphorase-type flavoprotein and cytochrome c or between cytochromes b and c. The chief evidence for the factor was based on the observation that BAL inhibited the oxidation of succinate or DPNH by cytochrome c but did not inhibit the oxidation of these compounds by methylene blue. The reaction carried out by a combination of diaphorase and Slater's factor (Scheme IV) therefore was equivalent to the reaction catalyzed by DPNH-cytochrome c reductase. However, since soluble, purified DPNH-cytochrome c reductases can be isolated from heart muscle and these enzymes are not affected by BAL (*23*), it was necessary to postulate two routes for DPNH oxidation in the Keilin and Hartree preparations (*48*). There is additional evidence based on inhibition by antimycin A to indicate that more than one route for DPNH oxidation exists (*43, 49, 50*) in animal and plant tissues.

In the respiratory chain proposed by Slater (Scheme IV) the location of cytochrome b has provided the greatest difficulty. Cytochrome b was specifically excluded by Slater from the sequence in which DPNH was oxidized by O_2 (*41*). This exclusion, which was contrary to earlier work (*36*), was based on the observation that DPNH addition did not yield reduced cytochrome b. Since, however, succinate addition did reduce both cytochromes b and c, the electrons from oxidation of succinate were thought to enter the respiratory chain at the level of the new component, Slater's factor (*41*).

In contrast to this work is the observation by Chance (*51*) that the rate of reduction of cytochrome b is not adequate to permit it to function in the oxidation of succinate by the *non-phosphorylating* heart muscle preparation. However, there is evidence that cytochrome b is a member of the carrier sequence linking both DPNH and succinate oxidation to cytochrome c in intact *phosphorylating mitochondria* (*52*). Therefore Chance and Williams (*13*) suggest that in the non-phosphorylative Keilin-Hartree preparation Slater's factor is that part of the cytochrome b complex which is sensitive to inhibitors such as BAL. Slater's factor has recently been described (*15*) as a structural factor or a measure of the ability of cytochromes b and c to interact. Other authorities in this field believe that the roles played by cytochrome b and "factor(s)" which may link a reaction between cytochrome b and cytochrome c are not yet defined. Apparently these questions can be settled only after additional work (*14*).

b. Electron Transport Particle. Green and his associates have carried out studies on a submitochondrial unit which they have termed the electron transport particle (ETP) (*53–55*). Two types of ETP can be obtained from heart muscle mitochondria, either by homogenizing the particles in 8.5% sucrose, pH 8.5, or by alcohol fractionation in the presence of KCl. Certain assumptions regarding the physical structure of ETP in the mitochondrion have been made. The particles catalyze the oxidation of succinate and DPNH by molecular oxygen in reactions which are inhibited by cyanide and antimycin A. These particles therefore have properties in common with the Keilin and Hartree preparation but, in contrast, do not require added cytochrome c. The latter when added to ETP usually has no effect.

The ETP is considered to be the parent particle of two other particulate preparations known as succinic dehydrogenase complex (SDC) and DPNH oxidase. The ETP can be fractionated with desoxycholate into two components, a red and a green particle (*56*). The former will oxidize succinate (or DPNH) with cytochrome c or ferricyanide as acceptor, and contains, as determined spectrophotometrically, cytochromes b and c_1. It therefore appears similar to SDC, which can be obtained directly by treatment of beef heart mitochondria with alcohol (*57*). SDC catalyzes the oxidation of succinate by cytochrome c, ferricyanide and dyes. The green particle contains cytochromes b, c, and a and catalyzes the oxidation of DPNH by O_2 if cytochrome c is present. The green particle also catalyzes the oxidation of ferrocytochrome c.

Green's laboratory has also described a DPNH-oxidase which is derived from ETP and does not have the ability to oxidize succinate (*58*). The DPNH-oxidase has also been fractionated into a green and a red

component (*59*). The red component catalyzes the oxidation of DPNH by cytochrome c (cytochrome reductase) and the green possesses cytochrome oxidase activity.

Other attempts have been made to obtain particulate entities which contain the cytochrome respiratory chain relatively intact. Gamble and Lehninger exposed rat liver mitochondria to a variety of treatments involving mechanical and chemical disruption (*60*). Their procedures yielded particles capable of oxidizing succinate, β-hydroxybutyrate, and ferrocytochrome c by molecular O_2. From quantitative measurement of the amount of activity on the various sized particles, the authors concluded that organized respiratory structures were distributed throughout the mitochondria. Evidence that the subunit existed in a spectrum of sizes was obtained in these studies.

2. *Phosphorylative Particles*

a. Mitochondria. Studies on the localization of enzymatic activities within the cell have confirmed Claude's suggestion that the mitochondrion is the power plant of the cell (*61*). Although earlier workers had shown respiratory enzymes to be associated with the insoluble elements of the cell, much of our information results from the efforts of Hogeboom, Schneider, and their collaborators in this field (*62*). These workers demonstrated that cytochrome oxidase was contained almost exclusively in the mitochondrion (*63, 64*). The ability of mitochondria to catalyze the aerobic oxidation of numerous organic substrates establishes the fact that these particles contain the necessary components for transport of electrons from substrates to O_2. However, quantitative determination of the localization of the intact respiratory chain within the cell is clearly impossible because of the multicomponent nature of the chain.

In recent years Chance and his collaborators have provided the most detailed information concerning the cytochrome respiratory chain in phosphorylating mitochondria. This group has used special spectrophotometric procedures involving double beam and split beam recording spectrophotometers (*65*). The double beam instrument measures changes in the extent of oxidation of individual components of the respiratory chain as a function of time under different experimental conditions (*66*). The disappearance of dissolved O_2 in the reaction mixture may also be followed by the use of a vibrating platinum electrode (*65, 67*). In some cases rapid flow techniques (*65, 68*) have been introduced to obtain information on processes which are extremely rapid. The split beam spectrophotometer (*65, 69*) measures the difference in light absorption (the difference spectrum) as a function of

wavelength between oxidized and reduced biological samples. The degree of oxidation can be controlled by adding substrates, by depleting the dissolved oxygen in the sample, and by maintaining anaerobic conditions. This method has the advantage of detecting small changes in absorption of some components without interference by other strongly absorbing substances.

With these techniques Chance and Williams have observed the respiratory chain directly in intact mitochondria in which the process of hydrogen and electron transfer is tightly coupled to the phosphorylation of ADP. This is in contrast to the non-phosphorylative oxidation of DPNH and succinate catalyzed by the Keilin and Hartree muscle preparation. From spectral studies in which the mitochondria were exposed alternately to an excess of DPNH or O_2, and to anaerobic conditions, Chance and Williams concluded that cytochrome b functions in DPNH oxidation (*68*). This conclusion, which is supported by observations with intact yeast (*70*) and ascites tumor cells (*71*), is also in contrast to results obtained with the Keilin-Hartree preparation.

Information on the sequence of carriers in the respiration chain has also been obtained (*68*). One way in which this was done was by making O_2 available to mitochondria in which the carriers were in the reduced state and observing the order in which the different carriers became reoxidized. Under these conditions the terminal oxidase (cytochrome a_3) was first reoxidized, then cytochrome a, the next carrier, was oxidized and so on. The data obtained indicated that the chain has the composition shown in Scheme V.

$$\text{Substrate} \rightarrow \text{DPNH} \rightarrow \text{Flavoprotein} \rightarrow \text{Cytochrome b} \rightarrow \text{Cytochrome } c \rightarrow \text{Cytochrome a} \rightarrow \text{Cytochrome } a_3 \rightarrow O_2 \quad \text{(V)}$$

The position of succinic dehydrogenase in this scheme is less clear since purified succinic dehydrogenase does not contain cytochrome b (*72*). However, Chance (*13*) suggests that the dehydrogenase may be composed of flavoprotein and cytochrome b.

There is little doubt that a high degree of structural organization must exist in any tissue preparation which exhibits succinoxidase or DPNH-oxidase activity. At present, studies are being made of this organization as it exists in the mitochondrion. Components of the structure [succinic dehydrogenase (*72, 73*), succinic dehydrogenase complex (*57*), DPNH-cytochrome reductase (*23, 24*)] are being isolated and examined. There are several recent evaluations of our present state of knowledge (*39, 55, 74*).

It should be emphasized that there is not universal agreement regarding the composition of the respiratory chain shown in Scheme V

Hartree (*15*), for example, described the cytochrome pathway substantially as it is presented in Scheme IV above. In an appropriately titled article in the same volume in which the review by Chance and Williams (*13*) appears, Wainio and Cooperstein (*14*) discuss some of the controversial aspects of the cytochromes. Their scheme does not include cytochrome b specifically and does not differentiate between cytochromes a and a_3. These differences result from the fact that nearly all of the components of the chain are associated with the insoluble structures of the cell. For the purpose of the present paper these differences should not prove too great a disadvantage but should serve to emphasize that further modification may be necessary when more information is obtained. Although more information can be obtained in part by solubilization and purification of the components of the carrier chain, the value of direct observation of the respiratory chain in intact cells or in unmodified cellular components such as mitochondria should not be underestimated.

b. Submitochondrial Fragments. Lehninger and his associates have been successful in fragmenting mitochondria into smaller units which are capable of oxidative phosphorylation (*49, 75, 76*). The particles, estimated to be 1/3000 the size of mitochondria, were prepared by treating intact rat liver mitochondria with digitonin. The fragments catalyze the aerobic oxidation of β-hydroxybutyrate, DPNH, and succinate. They do not, however, oxidize any other member of the Krebs cycle, and they do not oxidize fatty acids. Of major importance is the ability of these particles to carry out oxidative phosphorylation. P/O ratios approaching 3 were obtained with β-hydroxybutyrate as substrate (*75*).

The oxidation of DPNH by the digitonin particles is only partly inhibited by antimycin A, although this compound completely inhibits the aerobic oxidation of β-hydroxybutyrate. The authors conclude that DPNH added to the digitonin fragments is oxidized by a non-phosphorylative DPNH-cytochrome c reductase (*23*) activity present in their preparations. Spectral studies have shown that the digitonin particles contain a flavoprotein and cytochromes b, c, a, and a_3. However, no attempt was made to establish the sequence of the carriers as they function in electron transfer.

Other workers have been successful in preparing submitochondrial fragments which are capable of oxidative phosphorylation. In Green's laboratory, alcohol fractionation of heart muscle sarcosomes has produced a phosphorylating electron transport particle (PETP) which oxidizes β-hydroxybutyrate, DPNH, succinate, and other Krebs cycle intermediates (*77, 78*). P/O ratios in excess of 2 have been obtained

during the oxidation of α-ketoglutarate, pyruvate (plus malate), and glutamate. No phosphorylation occurred during the oxidation of succinate or DPNH.

Two other laboratories have recently reported the fragmentation of rat liver mitochondria by sonic oscillations (*79, 80*). These particles appear to be more highly organized units than those described by Lehninger or than PETP, since they catalyze the oxidation of Krebs cycle substrates in addition to succinate and DPNH and support phosphorylation during oxidation of all of the substrates. P/O ratios of approximately 1.0 were observed during the oxidation of succinate, DPNH, glutamate, β-hydroxybutyrate, isocitrate, and proline. The submitochondrial fragments obtained by these different procedures would therefore appear to be units in which the cytochrome respiratory chain and the phosphorylation mechanisms are relatively intact. They may represent subunits of the structure of the mitochondrion itself.

D. Distribution of the Cytochrome Chain

1. *Evidence Based on Sequential Reduction and Oxidation of Carriers*

The information on the distribution of the cytochrome respiratory chain in nature consists of several types of evidence. Perhaps the most complete evidence for the functioning cytochrome chain consists of those studies in which sequential reduction and oxidation of the several carriers of the chain have been observed. Unfortunately such studies have been conducted on only a few tissues. Thus, the respiratory sequence shown in Scheme V has been observed directly in mitochondria from rat and guinea pig liver and in the Keilin and Hartree succinoxidase particles. Similar observations have been made in intact yeast cells and in ascites tumor cells (*13*).

Lundegardh (*81*) examined the cytochromes in roots of wheat plants by spectrophotometric techniques and concluded that the route for electron transfer through the cytochromes is from b to c to a. These conclusions were based on the type of study employed by Chance and Williams in which the sequence of reduction is observed directly. Recently Lundegardh has examined bakers' yeast (*82*).

2. *Evidence Based on Oxidation of Succinate or the Reduced Pyridine Nucleotides*

Much of the evidence for the distribution of the cytochrome respiratory chain consists of studies on the oxidation of succinate and DPNH by cell-free tissue preparations. The oxidation of succinate and DPNH by a tissue preparation obviously is only indirect evidence that the cyto-

chrome respiratory chain is functioning. By combining these experiments with studies of the action of inhibitors, more positive evidence for the chain can be obtained. The tissue preparations studied range in degree of homogeneity and structural organization from crude homogenates to isolated mitochondrial and submitochondrial fractions. Mitochondrial preparations probably constitute the largest group of preparations which have been examined and, in most mitochondrial studies, the aerobic oxidation of succinate will have been observed. The DPNH-oxidase activity of mitochondria has been less frequently examined. However, the DPNH-oxidase activity of rat liver mitochondria has been extensively investigated with respect to the ability of this reaction to support oxidative phosphorylation (*83*).

It would be impossible to list all the species or tissues from which mitochondrial preparations have been obtained and studied. It would be reasonably safe to assume that, with study and some care, mitochondria can be isolated from any higher plant or animal species and that such preparations will contain succinoxidase or DPNH-oxidase activity.

In the past the heart, kidney, and liver of beef, horse, rat, and pig have served as sources of cell-free preparations which exhibit succinoxidase or DPNH-oxidase activity (*41, 45, 84–89*). More recently rat skeletal muscle (*90*) has been examined. DPNH-oxidase and succinoxidase activity has also been observed in the submitochondrial units represented by the Keilin-Hartree (*41, 42*) preparation, the electron transport particle (*53*), and DPNH-oxidase particle (*58*) obtained from ETP.

DPNH-oxidase activity has been observed in homogenates of the thoracic muscle of the house fly (*91*), the midgut of silk worm larvae (*92*), and the flight muscles of the adult silk worm (*92*). The oxidation of succinate has been examined in homogenates of flight muscle of blow flies (*93*). Both DPNH-oxidase and succinoxidase have been detected in homogenates of tetrahymena (*94, 95*). In the latter case the oxidations are atypical, however, since the reactions, although cyanide-sensitive, do not appear to involve cytochrome c.

The oxidation of succinate has been investigated in higher plants (*96–99*) and has been observed to occur in a large number of plant mitochondria (*15, 100, 101*). DPNH oxidation studies have been carried out with mitochondria from potato tubers (*102*), from lupine cotyledons (*103*), and with mitochondria and microsomes from the aroid spadix (*103a*). In the studies on potato mitochondria, Hackett observed nearly complete inhibition of DPNH-oxidase activity by antimycin A. In the presence of added cytochrome c, antimycin A inhibited the rate of oxi-

dation of DPNH only 40%. These results indicated that the added cytochrome made an alternate, antimycin-A-insensitive oxidation route available for DPNH oxidation in plant tissues. Lehninger (*49*) and associates drew similar conclusions in studies with submitochondrial fractions from rat liver and suggested that their results were due to the presence of DPNH-cytochrome c reductase in their fractions. The latter enzyme is insensitive to antimycin A (*104*).

Succinoxidase activity has been observed in particles (mitochondria) isolated from bakers' yeast (*105*). These particles in turn have served as a source for isolation of a soluble succinic dehydrogenase which closely resembles its counterpart in beef heart mitochondria (*106*).

The oxidation of succinate and DPNH in bacteria is also associated with a particulate fraction. However, there are some major differences between these particles and the mitochondria which can be isolated from cells of plants and animals. For example, the particles from *Azotobacter vinelandii* are much smaller than mitochondria (*107, 108*); the diameter of the bacterial particles is only about 1% of that of animal mitochondria. Secondly, the small bacterial particles from *Azotobacter vinelandii* oxidize only succinate without additions. This is in contrast to animal mitochondria, which can oxidize all of the intermediates of the Krebs cycle without supplementation. The rate of succinate oxidation by the bacterial particles is quite rapid, however. Recently a particle isolated from *Azotobacter vinelandii* has been described as having all of the properties of the electron transport particles prepared from beef heart mitochondria (*109*). But in the bacteria there is nothing resembling the mitochondrion, and the activities described above are localized in independent small particles. Other properties exhibited by these particles have been observed and their significance has been discussed (*107*).

In their studies on oxidative phosphorylation in particulate preparations of *Azotobacter vinelandii*, Rose and Ochoa observed succinoxidase activity (*110*). Succinoxidase has also been observed in particles from *Serratia marscescens* (*111*), *Mycobacterium phlei* (*112*), *Corynebacterium creatinovorans* (*113*), *Escherichia coli* (*114*), *Aerobacter aerogenes* (*115*), and *Bacillus stearothermophilus* (*116*).

DPNH oxidase activity has been observed in the particulate preparations from *Azotobacter vinelandii* (*107, 110*) and *Mycobacterium phlei* (*112*).

3. *Evidence Based on Distribution of Components of the Chain*

The major evidence for the cytochrome respiratory chain is based on the widespread distribution of components of the chain. Of all the

components, the greatest amount of information available relates to the occurrence of the cytochromes. The broad distribution of the cytochromes was first indicated by Keilin's studies (*5*). His work showed that the cytochromes were present not only in animal tissues, as MacMunn (*4*) had recognized, but were also present in yeast, insects, higher plants, and aerobic bacteria. Recently even the exceptions cited by Keilin, the anaerobic bacteria, have been shown to contain cytochromes. Thus a cytochrome of the c type was found in *Chlorobium limicola,* an obligate anaerobe and photoautotroph (*117*). Postgate (*118*) has purified a cytochrome with an unusually low oxidation-reduction potential ($E_0 = -0.205$, pH 7.0) from *Desulfovibrio desulfuricans,* a strict anaerobe. A sample of cytochrome, possibly containing several heme-proteins, was isolated from an obligate photosynthetic anaerobe, *Chromatium* (*119*). This protein fraction has properties similar to plant cytochrome f and to mammalian cytochromes c and b. Kamen and Vernon (*120*) have examined the cytochromes present in the nonsulfur purple bacteria and Vernon has isolated and partially purified cytochromes from the denitrifiers, *Micrococcus denitrificans* and *Pseudomonas denitrificans* (*121*). Of interest is the fact that *Streptococcus faecalis* (*16, 122*) and *Clostridium pasteurianum* (*123*) contain no hematin compounds. Instead, Dolin has shown the presence of flavoproteins capable of oxidizing DPNH by O_2 in *S. faecalis* (*124*).

In earlier work, cytochromes had been recorded among the fungi in *Neurospora crassa* (*125*), *Penicillium notatum* (*125*), *Ustilago* sp. (*126*), *Myrothecium verrucaria* (*127*), and *Aspergillus* sp. (*128*). In a late report, the absorption bands of cytochrome were detected in the mycelia of 45 fungal species by direct observation with a hand spectroscope (*129*).

The plant cytochromes have been the subject of a recent review (*15*). The distribution of pigments is broad. With plants, however, there is some reason to believe that certain of the cytochromes perform unique functions in addition to their role in respiration. Thus, Lundegardh (*130*) has proposed a scheme for ion absorption which is intimately associated with cytochromes a_3, a, c, and b of the respiratory chain. In addition, Hill and Hartree (*131*) have commented on the possibility that a new cytochrome, cytochrome f, which is present in high concentration in chloroplasts, may participate in photosynthesis. Recently the photooxidation of cytochrome c (*132*) has been observed in chloroplasts. Because ferrocytochrome f is oxidized by ferricytochrome c, this constitutes a mechanism for linking the reducing power of ferrocytochrome f to the reduction of the oxidizing component ("OH") formed in the photolysis of H_2O. Two other plant pigments, cytochromes b_3 and b_7,

may account for the cyanide-resistant respiration encountered in some plant tissues (*15*). Both of these cytochromes are autoxidizable, and the former is reduced by DPNH and TPNH. They therefore provide a means for linking the oxidation of respiratory substrates to O_2 in a manner independent of the cyanide-sensitive cytochrome oxidase.

III. Oxidative Phosphorylation

A. Early Studies

The cytochrome respiratory pathway has been described in detail because of its intimate association with the process of oxidative phosphorylation. Kalckar (*133*) was among the first to show that the conversion of inorganic phosphate to sugar phosphates in tissue extracts could be made dependent upon respiration. In subsequent studies he demonstrated that the addition of substrate to such extracts increased both the phosphorylation and the oxygen uptake.

Somewhat later Belitzer and Tsibakowa (*134*) reported that, in studies carried out in muscle minces, approximately 2 moles of phosphocreatine were formed for every atom of O_2 consumed by the oxidation of different substrates. This observation of the ratio of phosphorus esterified to oxygen consumed (the P/O ratio) was an extremely important finding, for it demonstrated that more than one phosphorylative step could occur in the transfer of a single pair of electrons from substrate to oxygen. Although one of the phosphorylation steps observed in their studies might well have been associated with substrate oxidation, the findings of Belitzer and Tsibakowa implied that at least one phosphorylation step was associated with electron transfer through the cytochrome system.

The significance of these findings was recognized by others working in the field at that time and they were confirmed. Interest centered in the phosphorylation associated with the oxidation of pyruvate because this compound, an end product of the glycolytic sequence, could be oxidized to completion by means of the Krebs cycle. In a detailed paper, Ochoa (*135*) examined pyruvate oxidation in heart preparations and observed P/O ratios in the range of 2.0. Phosphate losses were estimated as high as 60% due to the very active dephosphorylation of ATP that occurred. Therefore, Ochoa corrected the value of 2.0 to 3.0. Thus, 15 phosphorylations representing the conservation of perhaps as much as 150 kcal. were believed to be associated with the oxidation of pyruvate. Ochoa also clearly stated the problem of localization of the sites of phosphorylation in the electron transport scheme and made the first attempt to demonstrate phosphorylation associated with the oxida-

tion of DPNH. The oxidation of DPNH resulted in large oxygen uptake, but there was no phosphorylation. For a more detailed discussion of the older work in oxidative phosphorylation, the reader is referred to the reviews by Hunter (*136*) and by Lehninger (*137*).

B. Energy Relationships

Belitzer (*134*) observed that the difference in oxidation-reduction potential between substrates and O_2 was sufficient to permit the esterification of several moles of inorganic phosphate for each oxidation equivalent. Moreover, in 1939 the cytochrome pathway was known in some detail and it was recognized that the electron transfer passed through a series of carriers in stepwise manner. Thus Belitzer proposed that each of those steps in which DPNH, flavoprotein or cytochrome was oxidized in turn by the next carrier represented an energy-releasing step which could be linked to phosphorylation. Although these relationships have been thoroughly discussed elsewhere (*13, 137, 137a*) they will be reviewed briefly again.

The oxidation-reduction potentials (E_0) at pH 7.0 for the carriers of the cytochrome chain are given in Table I. Since a potential differ-

TABLE I
OXIDATION-REDUCTION POTENTIALS[a]

	E_0 volts	E_h
Pyridine nucleotide	−0.32	−0.32
Flavoprotein	−0.06	−0.03
Cytochrome b	0.00	+0.04
Cytochrome c	+0.26	+0.33
Cytochrome a	+0.29	+0.37
Oxygen	+0.82	+0.80

NOTE: E_0 is the standard oxidation-reduction potential at pH 7.0. E_h is the oxidation-reduction potential at pH 7.0 corrected for the ratio of the oxidized carrier to reduced carrier existing in intact mitochondria.

[a] The data in Table I are reproduced from a table prepared by Chance and Williams (*13*).

ence of 1.0 volt is equal to about 46 kcal., the transfer of one pair of electrons from reduced pyridine nucleotides to oxygen should be equal to approximately 52 kcal. This is adequate to allow for the maximum P/O ratios of 3 which have been observed during the oxidation of the pyridine nucleotides. Of greater interest is the potential difference between adjacent carriers in the scheme, since the magnitude of this figure may determine whether enough energy is released at that point to provide for the synthesis of a high-energy phosphate bond. For example,

the difference between the standard potentials for cytochromes a and c is only 0.03 volts (*13, 14*). This suggests that the one phosphorylation known to occur between cytochrome c and O_2 may occur between cytochromes a and a_3 or between cytochrome a_3 and O_2. Chance (*13*) has recently emphasized that calculations of this type must take into account the actual concentrations of products and reactants. Such information has been provided by these authors, and the E_h for the observed steady-state ratios are also given in Table I. However, since the ratios are not far from one, except for cytochrome a_3, the differences between E_0 and E_h are small.

C. Cellular Localization of Oxidative Phosphorylation

The earlier studies on oxidative phosphorylation were carried out with muscle minces (*134, 138*), "extracts" of kidney (*139*) or heart (*135*), and brain (*140*) homogenates. These types of preparations had to be freshly prepared each day, and little success was observed in reactivating preparations which had lost their activity. With the development of methods of isolation of mitochondria and examination of their enzymatic properties, these particles were shown to contain the necessary enzymes to support oxidative phosphorylation (*141–144*). As a result of using mitochondria, the phosphorylation processes were separated from side reactions catalyzed by soluble enzymes present in the cell cytoplasm.

Recently phosphorylative submitochondrial fragments have been obtained by treating mitochondria with digitonin (*75*) or alcohol (*78*), or by sonic vibrations (*79, 80*). Some of these particles will carry out phosphorylation during the oxidation of β-hydroxybutyrate, DPNH, succinate, and other Krebs cycle acids with P/O ratios in the range of 1 to 2. The digitonin and alcohol particles however support phosphorylation only during oxidation of β-hydroxybutyrate. The phosphorylation reactions are affected by uncoupling agents in a manner similar to that exhibited by the intact mitochondrion. However, oxidation of DPNH by the particles obtained by sonic treatment is much more sensitive to the inhibitor, antimycin A. All of these particles, regardless of manner of preparation, would seem to constitute simpler systems in which to study oxidative phosphorylation.

In bacteria the phosphorylation processes are associated with an even smaller particle (*107*).

D. Methodology

To determine the P/O ratio associated with the oxidation of an organic substrate, one wishes to measure the amount of inorganic phosphate which is esterified and the amount of O_2 uptake which occurs

simultaneously. In the absence of complicating factors, the former is equivalent to the amount of inorganic phosphate which disappears from the reaction mixture during a given period of time. This quantity can be determined by measuring the difference between the inorganic phosphate present at the beginning and at the end of the experiment. O_2 uptake during this period can be observed manometrically. Phosphatases which catalyze the hydrolysis of ATP will interfere, and lower P/O ratios will be observed. Ochoa (*140*) introduced the use of glucose and hexosemonophosphate as acceptors of phosphate from the adenylic acid system, since the phosphorylated sugars appeared to be less susceptible to phosphatase action. Fluoride was also added to the reaction mixtures to inhibit phosphatase action.

With the use of mitochondria (*145, 146*) and other particulate preparations (*141, 143*) in studies on oxidative phosphorylation, side reactions that are due to soluble enzymes present in homogenates become less significant. However, it is necessary to add hexokinase to the reaction mixtures (*146–149*) if glucose is employed as a phosphate acceptor from ATP (*141, 142*). ADP can serve as acceptor and under these conditions ATP will accumulate. In experiments of this sort fluoride addition inhibits ATP-ase activity and higher P/O ratios are obtained (*135, 145*).

A typical reaction mixture for studying oxidative phosphorylation would therefore contain mitochondria or some other source of the necessary enzymes, inorganic phosphate, Mg^{2+}, AMP or ADP, F^-, and substrate. Depending on the type of preparation or the substrate being studied, DPN^+ and/or cytochrome c might be added. In those experiments where the final phosphate acceptor is glucose, both glucose and hexokinase would be added to the reaction mixture. Only catalytic amounts of ADP are required.

The substrates most frequently employed in studies of this sort are the intermediates of the Krebs cycle. In addition the oxidation of glutamate (*141, 146*), butyrate (*141*), other fatty acids (*150*), and proline (*141*) are known to support oxidative phosphorylation. Another substrate which has been extensively used is β-hydroxybutyrate (*145*).

Slater introduced a valuable modification of the procedure for determining P/O ratios (*151*). He proposed measuring the amount of glucose-6-phosphate formed in the presence of a glucose-and-hexokinase trap rather than the disappearance of inorganic phosphate. Since sensitive and precise methods are available for determining extremely small amounts of glucose-6-phosphate (*152*), the phosphorylation process can be followed accurately at the beginning of the experiments when the disappearance of inorganic phosphate is small.

Studies on the localization of the sites of phosphorylation in the

cytochrome respiratory pathway have stimulated development of methods for measuring extremely small amounts of phosphate and respiration. In the former case, Lehninger (*153*) developed a method in which oxidative phosphorylation is carried out in the presence of radioactive inorganic phosphate. The inorganic phosphate is then extracted quantitatively as the phosphomolybdate in isobutanol-benzene and the radioactivity in the esterfied fraction is determined.

For determining the amount of oxidation which has occurred, Lehninger followed the reduction and oxidation of cytochrome c spectrophotometrically. In measuring phosphorylation occurring at oxidation levels between DPNH and cytochrome c, the reduction of the hemeprotein was measured and its reoxidation prevented by cyanide. For phosphorylation occurring between cytochrome c and oxygen, the amount of ferrocytochrome c oxidized was determined.

Krebs *et al.* (*154*) also employed P^{32} in measurements on oxidative phosphorylation. In their studies these authors measured the rate of incorporation of P^{32} into ATP under conditions where steady state concentrations of the inorganic phosphate and ATP existed.

Chance and Williams (*67*) have also introduced a new method for measuring the amount of phosphorylation associated with O_2 uptake. In their studies they employed mitochondria in which the phosphorylation process is tightly coupled to the oxidation processes, and they followed the concentration of oxygen in the reaction mixture with a vibrating platinum electrode. In the presence of excess substrate (β-hydroxybutyrate) the ADP present in the particles was phosphorylated and the O_2 consumption was retarded. Then, when small amounts of ADP were added, the oxygen content, as measured by the O_2 electrode, decreased a definite amount. The P/O ratio (or more accurately the ADP/O ratio) was then calculated from the ADP added and O_2 consumed.

E. P/O Ratios

1. *Oxidation of Krebs Cycle Substrates*

Much of the energy available in organic metabolites is released to the organism when acetyl-CoA is oxidized through the reactions of the Krebs cycle. Because Ochoa's (*135*) studies on rat heart extracts suggested that the average number of phosphorylation steps per atom of oxygen consumed during pyruvate oxidation was 3.0, it was of interest to determine which of the reactions of the cycle gave rise to oxidative phosphorylation and the number of phosphorylation steps which could be obtained.

It is now apparent that each of the four oxidation steps of the cycle can support phosphorylation. However, only one of the steps can be clearly isolated, specifically the oxidation of α-ketoglutarate. In order to determine the maximum P/O ratio which can be obtained, it is necessary to confine the reaction to a single oxidation step. In the case of α-ketoglutarate, the further oxidation of succinate can be effectively inhibited by the addition of malonate.

P/O ratios for oxidation of α-ketoglutarate have been obtained which are significantly greater than 3.0 (*146–149, 154*). This means that the number of phosphorylations which occur during oxidation of α-ketoglutarate is 4.0. Although objections were raised in favor of the lower value of 3.0 (*155*) in heart tissue, the question has now been settled in favor of 4.0 (*137, 146 148*). The probable number of phosphorylations associated with the other oxidation steps of the cycle are as follows: succinate, 2 (*141, 146, 148, 154*); malate, 3 (*137, 141, 148*); and citrate, 3 (*137, 141, 146*). In addition, the oxidation of pyruvate to acetyl CoA undoubtedly will give rise to 3 high-energy phosphate bonds (*137, 141, 146, 156*). Glutamate oxidation appears to proceed aerobically with P/O ratios approaching 3.0 (*141, 146, 148*).

2. *Oxidation of Reduced Pyridine Nucleotides*

Work in Lehninger's laboratory was the first to show conclusively that phosphorylation reactions occurred at sites along the cytochrome respiratory chain between the pyridine nucleotides and oxygen. Experiments with radioactive orthophosphate revealed incorporation of phosphate into an acid-labile organic phosphate (*157*), and a P/O ratio of 1.89 (uncorrected) was observed with the aerobic oxidation of DPNH by rat liver mitochondria (*158*).

Some interesting observations relating to the oxidation of external DPNH were made in these experiments. The oxidation of DPNH did not occur unless external cytochrome c (1.5×10^{-4} *M*) was added; on the other hand the oxidation of β-hydroxybutyrate and fatty acid by the same preparation was increased only slightly by the addition of cytochrome c. Moreover, higher P/O ratios with DPNH as substrate were obtained with mitochondria which had been pretreated with H_2O before their phosphorylating ability was measured. Such pretreated particles required much lower concentrations of cytochrome c for maximal oxidation rates. These results are best interpreted as showing that pretreatment can greatly increase the permeability of mitochondria to external DPNH and permits access of DPNH to internal sites of phosphorylation (*137, 159*).

The fact that chemically reduced DPNH can support oxidative

phosphorylation when added to mitochondria is of considerable importance (*158*). This observation implies that DPNH formed in extramitochondrial reactions catalyzed by soluble dehydrogenases can in turn be oxidized by the respiratory chain of the mitochondria to support phosphorylation. If this is possible, then the oxidation of substrates by pyridine-nucleotide-specific soluble enzymes outside the mitochondria may provide additional energy sources for the cell. The most important single example of an extramitochondrial enzyme which might function in this manner is the glyceraldehyde-3-phosphate dehydrogenase.

It should be emphasized that, in earlier studies, it was necessary to subject mitochondria to hypotonic treatment or to add cytochrome c (*158*) before the oxidation of external DPNH would support oxidative phosphorylation. However, Maley (*160*) has recently reported the oxidation of DPNH by rat liver mitochondria in experiments in which P/O ratios in excess of 2.0 were obtained.

Experiments of the sort just described are not comparable to conditions existing within the cell, for in the latter case DPNH would be generated in low concentration and immediately reoxidized by the respiratory chain. Lehninger suggested that the concentration of DPNH may determine whether phosphorylation will occur. He attempted to reproduce conditions in the cell by coupling the reduction of DPN^+ by crystalline alcohol dehydrogenase (*159*) to reoxidation by rat liver mitochondria. Essentially no phosphorylation was observed. However, in other experiments in which triosephosphate dehydrogenase was utilized as the DPNH-generating system, phosphorylation over and above that due to the substrate phosphorylation by the dehydrogenase did occur (*159*). The difference in the phosphorylating ability of the two coupled dehydrogenase systems was explained by the differences in equilibrium of the two systems. With triosephosphate dehydrogenase, approximately 90% of the total DPN was in the reduced form. In the case of alcohol dehydrogenase, less than 2% of the coenzyme was reduced. These experiments and their significance in the general economy of the cell have been extensively discussed (*137*, *159*).

Although the oxidation of DPNH will support the formation of 3 high-energy phosphate bonds, there is little evidence to indicate that TPNH can function in the same way. Instead, Kaplan *et al.* (*161*) have provided data indicating that the oxidation of TPNH through the electron transport system of rat liver mitochondria does not lead to oxidative phosphorylation. Only when the oxidation of TPNH is mediated through the enzyme transhydrogenase (*162*) and DPN^+ is there evidence for phosphorylation, the oxidation of DPNH formed in the transhydrogenase reaction (Reaction 6) yielding high-energy phosphate.

$$TPNH + DPN^+ \rightleftharpoons DPNH + TPN^+ \qquad (6)$$

Other workers have demonstrated that the oxidation of isocitrate by a TPN^+-linked dehydrogenase in rat liver mitochondria proceeds with a P/O ratio of approximately 1.0 (*163*). The evidence indicates that the single phosphorylation step occurs when ferrocytochrome c, reduced in the presence of TPNH and cytochrome c reductase, is oxidized by molecular O_2. This mechanism provides a means for obtaining some of the potential energy available in TPNH, but the process is much less efficient energetically than the process in which DPN^+ is reduced through the enzyme transhydrogenase.

3. *Oxidation of β-Hydroxybutyrate*

Before highly purified DPNH was available, Lehninger's group utilized the oxidation of β-hydroxybutyrate to obtain information concerning the phosphorylation processes coupled to pyridine nucleotide oxidations (*159*). The oxidation of β-hydroxybutyrate is a single step oxidation catalyzed by a DPN^+-requiring dehydrogenase. One of the products formed, acetoacetate, was metabolically inert in their system. The DPNH formed was reoxidized by the respiratory chain. Various factors (Mg^{2+}, arsenite, dinitrophenol) which were known to influence oxidative phosphorylation with DPNH also influenced phosphorylation associated with β-hydroxybutyrate to the same extent. There is no evidence to indicate that phosphorylation might occur at the substrate level between substrate and DPN^+ (*145*). P/O ratios in excess of 2.0 have been obtained in the oxidation of β-hydroxybutyrate both with intact mitochondria (*146*) and with submitochondrial fractions (*75*). These observations confirm the conclusion that 3 phosphorylations do occur in the respiratory chain between DPNH and O_2.

F. Sites of Phosphorylation

In recent years data has accumulated which permits some localization of the sites of phosphorylation along the respiratory chain. One site was definitely located when Friedkin and Lehninger (*157*) and Judah (*164*) observed oxidative phosphorylation during the oxidation of ascorbic acid. In mitochondrial suspensions containing cytochrome c, ascorbate reduces the heme-protein nonenzymically. Reoxidation of ferrocytochrome c aerobically results in phosphorylation. Maley and Lardy (*165*) and Lehninger *et al.* (*166*) have obtained P/O ratios approaching 1.0 for this reaction with rat liver mitochondria. Since ascorbate is a nonspecific reductant for cytochrome c, other reducing agents should substitute for ascorbate. The authors report that cysteine and adrenaline would substitute, but that lower P/O ratios were observed. This could be due to the fact that oxidation products of these compounds inhibit phosphorylation.

More recently, Nielsen and Lehninger (*153*) have re-examined the amount of phosphorylation coupled to the oxidation of ferrocytochrome c. Ferrocytochrome c was used in stoichiometric amounts rather than in catalytic quantities. As a result, methods were employed for measuring millimicromolar amounts of esterified phosphate and cytochrome. In these experiments the highest P/O ratio was 0.86, in good agreement with earlier values. In experiments of this type, the P/O ratio is equal to the expression:

$$\frac{\text{m}\mu\text{moles phosphate taken up}}{0.5\ \text{m}\mu\text{moles ferrocytochrome c oxidized}}$$

Similar values were obtained using a submitochondrial fraction from rat liver particles (*76*). For some time Slater questioned the existence of a phosphorylation step between cytochrome c and oxygen (*155*), but this has now been conceded (*167*).

With regard to the region of the respiratory chain between DPNH and cytochrome c it was known that phosphorylation occurred when ferricytochrome c (*168*) or ferricyanide was the oxidizing agent (*141, 146, 164*). The point at which ferricyanide reacts with the chain is not known, but Copenhaver and Lardy concluded that at least one phosphorylation occurred between the substrate and the point in the respiratory chain which is sensitive to antimycin A. Again using methods for measuring millimicromolar amounts of esterified phosphate, Borgström (*168a*) measured the phosphorylation which occurred during the oxidation of β-hydroxybutyrate by ferricytochrome c. The reduction of ferricytochrome c was measured and its reoxidation prevented by the presence of cyanide in the reaction mixture. The average P/O ratio observed was 1.51, confirming the maximum value of 2.0 expected. In later studies with submitochondrial fractions, Devlin and Lehninger (*49*) observed P/O ratios no greater than 1.0 for the oxidation of β-hydroxybutyrate by ferricytochrome c and concluded that this lower value represented a partial failure of the phosphorylation mechanism in these small particles.

In summarizing the preceding material, it appears that two phosphorylation steps occur somewhere between DPNH and cytochrome c and that one exists between cytochrome c and O_2. However, if the cytochrome respiratory path has the composition shown in Scheme V, more precise localization is desirable. Chance and Williams (*31, 169*) have discussed their approach to this problem and have defined "cross over points" as points in the respiratory chain where, as a result of some experimental condition, all of the carriers on one side of the point become oxidized and all of the carriers on the other are reduced. For example, when antimycin A is added to respiring mitochondria, cyto-

chromes a_3, a, and c become oxidized while cytochrome b, the flavoprotein of the chain, and pyridine nucleotide become reduced. Thus, antimycin A is believed to be reacting either with reduced cytochrome b or oxidized cytochrome c to prevent these two carriers from interacting.

In tightly coupled mitochondria, electron transfer becomes inhibited when the supply of phosphate acceptor (ADP) is depleted. Under these conditions, Chance and Williams have observed a cross over point between cytochrome c and cytochrome a. When low concentrations of azide are present, another cross over point occurs between cytochromes b and c. Finally, although there is extensive reduction of all the components between substrate and cytochrome in these mitochondria when the supply of ADP becomes limiting, it is found that DPNH is more than 99% reduced. Since this degree of reduction is far more than that of the other carriers, Chance and Williams proposed that a third cross over point exists between DPNH and flavoprotein. The cross over points are considered sites of energy conservation or oxidative phosphorylation (see Section III, G).

In the vast majority of the studies on phosphorylation associated with the respiratory chain pyruvate, one of the substrates of the Krebs cycle, cytochrome c, or DPNH has served as the hydrogen donor. In addition β-hydroxybutyrate (*145*, *146*), glutamate (*141*, *146*), proline (*141*), and fatty acids (*141*, *150*) when added to mitochondria will support oxidative phosphorylation. The dehydrogenases for these reactions are known to be localized in the mitochondrion, and the dehydrogenases for β-hydroxybutyrate, glutamate, and thioesters of β-hydroxy fatty acids are known to require pyridine nucleotides. In the case of the acyl-CoA dehydrogenases which catalyze the oxidation of thioesters of the fatty acids, the respiratory sequence presumably would be entered at the flavoprotein level and two phosphorylations would occur. There is no direct experimental evidence on this point, however.

The question of whether substrates other than these can give rise to oxidative phosphorylation is, of course, of interest. If a dehydrogenase for the substrate in question is localized on the mitochondria, the oxidation of the substrate may well yield high-energy phosphate. Moreover, the results of previous work suggest that, under proper conditions, soluble dehydrogenase systems may also support phosphorylation. The number of reactions investigated is small, however, and only the triosephosphate dehydrogenase system functioned successfully in this regard.

G. Mechanism of Oxidative Phosphorylation

Although the phosphorylations associated with the respiratory chain have been extensively studied during the past decade, there is little understanding of the precise mechanism accounting for the formation

of ATP (*13, 137, 170, 171*). As a result theories have been proposed by the leading workers in this field and these have undergone modification or elaboration. Those proposed by Slater (*170, 172, 173*) and Lehninger (*137, 174*) differ in one respect from that advocated by Chance (*13, 175*). The theories of Slater and Lehninger contain the following mechanism: Two carriers, *A* and *B*, adjacent to each other in the respiratory chain undergo oxido-reduction in the presence of a third substance *I*. The nature of *I* is unknown, but it may be an enzyme. Prior to the oxidation of AH_2 by *B*, the reduced carrier AH_2 reacts with *I* (Reaction 7).

$$AH_2 + I \rightleftharpoons AH_2{-}I$$

Then during oxidation-reduction, the linkage between the carrier and *I* is raised to a high energy level (Reaction 8).

$$AH_2{-}I + B \rightleftharpoons A\sim I + BH_2$$

A portion of the free energy change of the reaction is conserved in the high-energy bond of $A\sim I$, the energy-rich complex. The analogy between this reaction and the oxidation of glyceraldehyde-3-phosphate is clear (*176*). In this proposal, the respiratory carrier participating in the high-energy complex is oxidized. Wadkins and Lehninger have recently provided data which they interpret in support of this proposal (*177*).

Next, the high-energy form of the carrier can react with an additional component *X* to form a new energy-rich compound ($X\sim I$) and regenerate the respiratory carrier (Reaction 9).

$$A\sim I + X \rightleftharpoons X\sim I + A$$

Although the original mechanism proposed by Slater (*170*) did not provide for *X*, kinetic evidence supplied by Chance and Williams (*13, 169, 178*) indicated that the respiratory carriers do not react directly with ADP or inorganic P and that one or more intermediates such as $X\sim I$ are involved. Intermediates are also indicated by the work of Slater (*172*) and Cohn (*179*).

In the final step, Slater suggests that $X\sim I$ can react with ADP and P_i to yield ATP (*172*) (Reaction 10).

$$X\sim I + ADP + P_i \rightleftharpoons ATP + I + X$$

The analogy between this reaction and the reaction catalyzed by succinic thiokinase is apparent (*180*) (Reaction 11).

$$\text{succinyl-CoA} + GDP + P_i \rightleftharpoons \text{succinate} + GTP + CoA$$

Lehninger's theory differs from the above in that he proposes reversible phosphorolysis of $A\sim I$ instead of Reaction 9. This yields

energy-rich phosphorylated form of *I* (Reaction 12) which then in turn can undergo transphosphorylation with ADP to form ATP (Reaction 13).

$$A\sim I + P_i \rightleftharpoons P\sim I + A \quad (12)$$
$$P\sim I + ADP \rightleftharpoons ATP + I \quad (13)$$

(In Lehninger's scheme, *I* is replaced throughout by C.)

The mechanism proposed by Chance differs from those of Lehninger and Slater in that the high-energy form of the carrier is *reduced* ($BH_2\sim I$) and not oxidized (*13, 181*). The evidence for this has been summarized, but the most important argument is the finding that DPNH (and presumably other reduced carriers) exists in an inhibited form in mitochondria lacking phosphate acceptors (*66, 169*). In Chance's proposal, the oxidized carrier *B* would first react with *I* (Reaction 14) and then be reduced by the adjacent carrier (AH_2) in the respiratory chain (Reaction 15).

$$B + I \rightleftharpoons B\text{—}I \quad (14)$$
$$B\text{—}I + AH_2 \rightleftharpoons BH_2\sim I + A \quad (15)$$

Again, because of the evidence that intermediates exist between the high-energy form of the carrier and ADP (*13, 169, 178*), a reaction with *X* occurs to give another high-energy compound (Reaction 16).

$$BH_2\sim I + X \rightleftharpoons X\sim I + BH_2 \quad (16)$$

X then is continually regenerated according to Reactions 17 and 18 in the presence of adequate supplies of ADP and P_i.

$$X\sim I + P_i \rightleftharpoons X\sim P + I \quad (17)$$
$$X\sim P + ADP \rightleftharpoons ATP + X \quad (18)$$

Any theory of oxidative phosphorylation must account also for the actions of various compounds and cations in uncoupling the oxidation and phosphorylation processes. 2,4-Dinitrophenol is perhaps the best example of an uncoupling agent (*182*), although it is not the only substance known to act this way (*137*). Dinitrophenol and other uncoupling agents have been postulated to act by accelerating the hydrolysis of $X\sim I$ in the theories proposed above (Reaction 19) (*19, 173, 174*), or by

$$X\sim I + H_2O \overset{DNP}{\rightleftharpoons} X + I \quad (19)$$

combining with $X\sim I$ to liberate *X* (*13*). Either of these possibilities accomplishes the liberation of *X* from $X\sim I$ and dissociates the phosphorylation processes from electron or hydrogen transfer through the respiratory chain. Experimentally, dinitrophenol stimulates the liberation of phosphate from ATP; that is, it appears to accelerate ATP-ase activity (*183*). However, Lardy and Wellman consider the action of dinitrophenol to be indirect and suggest that its action is on some other high-

energy compound formed prior to ATP. Slater emphasizes that a stimulation of apparent ATP-ase activity will result if Reaction 10 is reversed and this reaction is coupled with Reaction 19.

$$\begin{array}{rl} \text{ATP} + I + X \rightleftharpoons X\sim I + \text{ADP} + \text{P}_i & \text{(10R} \\ X\sim I + \text{H}_2\text{O} \longrightarrow X + I & \text{(19} \\ \hline \text{ATP} + \text{H}_2\text{O} \longrightarrow \text{ADP} + \text{P}_i & \text{Sum} \end{array}$$

Cooper and Lehninger (*174*) also account for the action of uncoupling agents by a reversal of Reaction 13 and a coupling of Reaction 13R with the hydrolysis of the phosphorylated intermediate (Reaction 20).

$$\begin{array}{rl} \text{ATP} + I \rightleftharpoons \text{ADP} + \text{P}\sim I & \text{(13R)} \\ \text{P}\sim I + \text{H}_2\text{O} \rightarrow \text{P}_i + I & \text{(20)} \\ \hline \text{ATP} + \text{H}_2\text{O} \rightarrow \text{ADP} + \text{P}_i & \text{Sum} \end{array}$$

Thus, the "ATP-ase" activity stimulated by dinitrophenol is considered to "represent a hydrolytic dysfunction" (*184*) of the enzyme system catalyzing transfer of phosphate between ADP and a primary high-energy phosphate compound ($\text{P}\sim I$) formed during oxidative phosphorylation. In this connection Hulsmann and Slater have recently observed four different enzyme systems in heart mitochondria which accomplish the hydrolysis of ATP. They suggest that three of these, which are stimulated in varying degrees by dinitrophenol, are related to the three dinitrophenol-sensitive steps in the respiratory chain phosphorylation (*173*).

Thyroxine (*185*), dicumarol (*76, 184*), and Ca^{2+} (*186*) are among other substances known to affect oxidative phosphorylation. Although the exact manner in which dinitrophenol acts is not known, there is good evidence to support the belief that Ca^{2+} ions and thyroxine do not function in the same manner when they "uncouple" phosphorylation. For example, Ca^{2+} and thyroxine have been shown to cause swelling of intact rat liver mitochondria, whereas dinitrophenol and dicumarol inhibit spontaneous swelling of these particles (*187*). Also, dinitrophenol (*75*) and dicumarol (*75*) inhibit oxidative phosphorylation carried out by the submitochondrial particles of Lehninger, while thyroxine (*188*) and Ca^{2+} (*75*) do not. The effect of these and other uncoupling agents has been reviewed (*13, 46, 185*).

The exchange of ATP with inorganic phosphate labeled with P^{32} has been investigated in rat liver mitochondria (*189, 190*) and correlated with ATP-ase activity in submitochondrial particles (*174*). On the basis of these observations Cooper and Lehninger have elaborated the scheme proposed earlier by Lehninger (*137*) for oxidative phosphorylation. Their scheme, which has been described above, accounts for the role of ATP, ADP, and inorganic phosphate in the exchange reactions with

$P^{32}O_4^{3-}$. The exchange of H_2O^{18} with ATP and inorganic phosphate which is catalyzed by liver mitochondria during phosphorylation has also been examined (*179, 189, 190*).

H. Occurrence of Oxidative Phosphorylation

The occurrence of oxidative phosphorylation associated with electron transport through the cytochrome respiratory scheme is indeed widespread. However, the detailed studies on the mechanisms of oxidative phosphorylation have been carried out with mitochondria or other particulates from only a few tissues. These are rat liver (*69, 75, 146, 147*) and beef (*78*) cat, pig, or rat heart (*191*).

Krebs *et al.* with the use of $P^{32}O_4^{3-}$ have measured phosphorylation in respiring tissue suspensions from rat liver, sheep heart, and pigeon breast muscle (*154*). Mitochondria prepared from other tissues of rat have been examined (*192, 193, 194*), as have particles from sheep kidney (*195*), rabbit heart (*196*), pigeon skeletal muscle, and the liver of the Syrian hamster (*197*). In addition, oxidative phosphorylation associated with the oxidation of Krebs cycle substrates has been observed in such interesting organisms as snails (*198*), locusts (*199*), blowflies (*200*), houseflies (*201*), mosquitoes (*202*), and the parasitic helminth *Ascaris lumbricoides* (*203*). The finding with the helminth is especially interesting, since the cytochrome respiratory chain is believed not to function in this organism.

Oxidative phosphorylation was first demonstrated in higher plants by Millerd *et al.*, who isolated and studied particles from etiolated mung bean seedlings (*204, 205*). Although the properties of these particles resembled those of animal mitochondria, the P/O ratios obtained during oxidation of various Krebs cycle substrates never exceeded 1.0. Shortly following these studies other workers observed oxidative phosphorylation with particles from cauliflower buds (*206*) and avocado fruit (*207*) in which the P/O ratio exceeded 1.0. Particles have now been isolated from etiolated lupine cotyledons (*208*), sweet potato roots (*209*), cabbage head (*210*), etiolated pea stems (*211*), and pea leaves (*212*) which are just as efficient in carrying out phosphorylation as are animal mitochondria. Phosphorylation has also been demonstrated to occur with particles from soy bean hypocotyls (*213, 214*) and castor bean endosperm (*215*).

Ohmura (*216*) has observed oxidative phosphorylation during the oxidation of Krebs cycle substrates by a particulate fraction from green leaves. Although he concluded that these particles were chloroplast fragments, there is other evidence (*217*) to indicate that green leaves contain other particles which might be responsible for Ohmura's observa-

tions. Phosphorylation has also been observed in particles prepared from the water mold *Allomyces macrogynus* (*218*).

Oxidative phosphorylation has been observed in a few microorganisms. Chief among these is *Azotobacter vinelandii,* where phosphorylation was observed first in cell-free extracts (*219*). Later work involved the isolation and study of small particles from this organism (*107, 110*). The P/O ratios obtained with these preparations are low in comparison with those obtained with animal mitochondria; moreover, the phosphorylation is largely insensitive to dinitrophenol. Phosphorylation has also been obtained with extracts from *Mycobacterium phlei* and *Corynebacterium creatinovorans* (*112*). The P/O ratios in these studies were higher; values greater than 1.0 were obtained with several Krebs cycle substrates. The phosphorylations were uncoupled by dinitrophenol.

Cell-free extracts of *Alcaligenes faecalis* support oxidative phosphorylation during the oxidation of DPNH (*220, 221*). It is possible to fractionate the extract into three components, a particulate DPNH oxidase fraction and two soluble fractions, one of which is labile to heat. Recent work suggests that the heat-stable component may be a polynucleotide of the ribonucleic acid type (*220, 222*). Although low P/O ratios (0.1–0.3) have been encountered in these studies, there is evidence to suggest that fewer cytochrome components are present in the oxidation chain responsible for the oxidation of DPNH (*223*). This in turn implies that fewer points of conservation of energy exist in the respiratory chain of these organisms.

IV. Other Respiratory Pathways

The possible existence of respiratory chains not involving the cytochromes has been investigated to the greatest extent in plant tissues. Lack of information concerning the cytochromes in plants stimulated searches for other terminal oxidases. Enzymes (and their substrates) which transferred electrons to molecular oxygen were known to be widespread in plants. In addition, the fact that the respiration of some plant tissues is relatively insensitive to carbon monoxide or cyanide indicated that a terminal oxidase other than cytochrome oxidase was functioning in those tissues. The large amount of information on this subject has been described and evaluated in several reviews (*224–227*).

The studies on terminal oxidases in higher plants have coincided with experiments which sought to determine the relationship of these enzymes to processes such as growth, water uptake, and ion accumulation which require energy. For example, the growth of sections of pea epicotyls and *Avena* coleoptiles was inhibited 90% in $CO:O_2$ mixtures of 20:1 (*228*). This inhibition was completely reversed in light in the case of the

pea tissue, and approximately 60% reversed in the light in the case of *Avena* sections. Since the reversal of CO inhibition by light is considered specific for cytochrome oxidase, there is little doubt that the energy for growth in these experiments was provided in an oxidation pathway mediated by cytochrome oxidase. Similarly, the inhibition by CO of water uptake in potato slices (*229*) was light reversible, as was the CO-inhibited salt absorption of barley roots (*230*). In these few cases, therefore, there is sufficient evidence to implicate the cytochrome respiratory chain as the source of energy for these forms of work. The relationships between respiration and cellular work have been discussed by Laties in a recent, valuable paper (*231*).

If alternate pathways for oxidation do exist in some organisms, the question of the physiological significance of these oxidation pathways is important. Although a major role should be that of conservation of energy in the form of energy-rich compounds, there is little direct evidence to indicate that energy can be made available other than through oxidations mediated by cytochrome oxidase. Instead, the non-cytochrome respiratory chains may constitute a mechanism for dissipating reducing power represented by DPNH or TPNH and accumulated during some special activity of the organism. Or the chain may function to keep easily oxidized substances as glutathione, ascorbic acid, and phenols in the reduced state.

Many enzymes in addition to cytochrome oxidase catalyze reactions involving molecular oxygen. These enzymes have been classified and discussed in some detail (*232*). However, only a few of these catalysts can seriously be considered for a role in cellular respiration. These are the enzymes which directly or indirectly can participate in the oxidation of reduced pyridine nucleotides, flavoproteins, or cytochromes. Some of these are discussed briefly.

A. Ascorbic Acid Oxidase

The widespread distribution of ascorbic acid oxidase in higher plants was a major factor in assigning a tentative role in respiration to this enzyme. The enzyme has been extensively purified (*233*) and characterized, and has been implicated in the respiration of germinating wheat seedlings (*99*) and barley roots (*227, 234*). On the basis of the low affinity of the enzyme for O_2, however, Bonner has concluded that ascorbic acid oxidase cannot play a significant role in any tissues except those which are highly aerobic in nature (*224*).

In contrast to this, Mapson and Moustafa have stated that ascorbic acid and glutathione can function in a respiratory system which may account for as much as 25% of the respiration of pea cotyledons (*235*).

This system consists of reactions catalyzed by four enzymes: a dehydrogenase requiring TPN^+, glutathione reductase, dehydroascorbic acid reductase, and ascorbic acid oxidase (*236, 237*). Reactions 21–24 are catalyzed by these four enzymes, respectively:

$$\text{Substrate} + TPN^+ \rightleftharpoons \text{Oxidized substrate} + TPNH + H^+ \quad (21)$$

$$TPNH + H^+ + GSSG \rightleftharpoons TPN^+ + 2\ GSH \quad (22)$$

$$2\ GSH + DHA \rightleftharpoons GSSG + AA \quad (23)$$

$$AA + \tfrac{1}{2}\ O_2 \rightarrow DHA + H_2O \quad (24)$$

The oxidation of ascorbic acid (Reaction 24) can also be mediated through the phenol oxidases or cytochrome oxidase.

Although a fraction of the respiration of the pea seedling may be mediated through the respiratory chain described in Reactions 21 through 24, the question of the physiological significance of this respiration has been raised. The possibility of these oxidations being associated with phosphorylation is questionable, inasmuch as the oxidative enzymes are found mainly in the soluble fractions of the cell. If the oxidation-reduction potential (E_0) of the GSSG-GSH couple is +0.03 volt (*238*), the difference in potential between glutathione and the pyridine nucleotide is large enough to support phosphorylation in Reaction 22. However, attempts to demonstrate oxidative phosphorylation during the anaerobic reduction of oxidized glutathione by Krebs cycle substrates in the presence of avocado mitochondria were unsuccessful (*239*). Results such as these suggest that the function of the respiratory chain studied by Mapson and Moustafa is other than making energy available to the plant. There is apparently only one report of an energy-consuming process, that of ion absorption, being linked to ascorbic acid oxidase (*240*). Reactions 21 through 23 provide a means for maintaining glutathione and ascorbic acid in the reduced state. Since reduced glutathione is known to influence the enzyme activity of proteins containing sensitive sulfhydryl groups, this may be the major function of the enzyme system which accomplishes reduction of glutathione in living tissues (*236, 237*).

There is another way in which the oxidation of ascorbic acid can be linked to the oxidation of a reduced pyridine nucleotide. Several years ago Mathews (*241*) observed that a protein fraction from pea seeds catalyzed the oxidation of DPNH in the presence of ascorbic acid. Since the reaction was insensitive to cyanide, Mathews concluded that ascorbic acid oxidase was not involved in his studies. Subsequent work has shown that peas (*242, 243*), yeast (*244*), and other plants (*243*) contain an enzyme which will catalyze the oxidation of DPNH by a product formed by the action of O_2 on ascorbic acid in the presence of its oxidase (Reaction 24). Although dehydroascorbic acid is the final oxidation product when ascorbic acid is oxidized in the presence of its oxidase,

dehydroascorbic acid will not serve to oxidize DPNH. Therefore, an intermediate oxidation product "monodehydroascorbic acid" (a semiquinone) has been postulated (Reaction 25).

$$AA + \tfrac{1}{2}\,O_2 \xrightarrow{\text{Ascorbic acid oxidase}} \text{Monodehydroascorbate} \qquad (25)$$

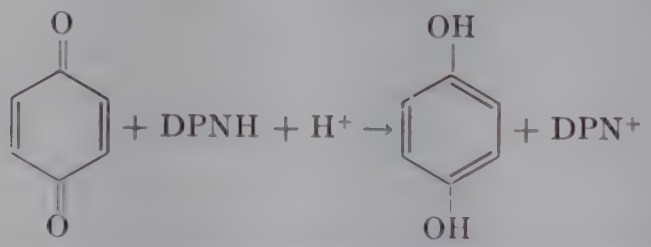
It is further postulated that the monodehydroascorbate in turn is reduced by DPNH in the presence of the enzyme found in yeast, peas, and other plants (Reaction 26).

$$DPNH + H^+ + \text{Monodehydroascorbate} \rightarrow DPN^+ + AA \qquad (26)$$

It is interesting to note that Cu^{2+} and Fe^{2+} can replace ascorbic acid oxidase in Reaction 25 in catalyzing the formation of the reactive intermediate. The significance, if any, of this reaction in plant respiration is unknown.

B. Phenol Oxidases

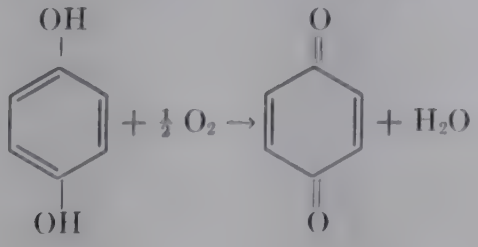
The phenol oxidases which catalyze the oxidation of phenolic compounds to their corresponding quinones are widely distributed in nature (*245, 246*). Quinones thus formed in turn can be reduced nonenzymically by the reduced pyridine nucleotides (*247, 248*) (Reaction 27). In addition, however, an enzyme, quinone reductase, has been shown to catalyze Reaction 27.



(27)

Quinone reductase is found in higher plants, fungi, yeast, bacteria, and in animal tissues (*249*). It has been extensively purified from pea seeds and characterized. The enzyme reacts with both DPNH and TPNH although at different rates, and with 6 quinones out of 14 tested (*248*). It is strongly inhibited by 2,4-dinitrophenol.

Reaction 27, when coupled with the oxidation of phenols (Reaction 28) catalyzed by the phenol oxidases, provides a means for oxidizing the reduced pyridine nucleotides aerobically through the mediation of quinone reductase and a naturally occurring quinone.



(28)

Because of the reactivity of 1,2-naphthoquinone in these reactions and the widespread occurrence of naphthoquinones in nature, Wosilait *et al.* speculated that these compounds may be naturally occurring carriers in the transfer of electrons from respiratory substrates to oxygen (*249*).

The possible role of the phenol oxidases in plant respiration has been discussed (*224, 226, 245, 249*). There is little evidence to support polyphenol oxidase as a terminal oxidase in plants, even through a carrier system between DPNH or TPNH and the quinones exists. The tissue which has been studied most is the potato tuber in which polyphenol oxidase is abundantly present. However, in 1950 Goddard and Holden demonstrated that there was a sufficient amount of cytochrome oxidase in homogenates of potato tuber to account for the respiration of the intact tissue (*250*).

Again, as with ascorbic acid oxidase, the affinity of the phenol oxidases for O_2 apparently is low, and it is suggested that this enzyme may instead be involved in electron transfer to some acceptor besides O_2 (*224*). The need for more detailed studies on the properties of the purified enzyme has been emphasized.

C. Flavoproteins

The role of flavoproteins in the respiration of the facultative anaerobe *Streptococcus faecalis* has been examined in some detail by Dolin (*122, 124, 251, 252*). The electron transfer in this organism is of interest since this bacterium is lacking in the cytochromes and catalase. The absence of catalase is particularly interesting, since this introduces the possibility of H_2O_2 accumulation as a result of flavoprotein interaction with O_2. Under anaerobic conditions the oxidation of DPNH in these organisms is coupled to the reduction of pyruvate with the formation of lactate.

Cell-free extracts of *S. faecalis* contain two enzymes which catalyze Reactions 29 and 30.

$$\text{DPNH} + \text{H}^+ + \text{O}_2 \rightarrow \text{DPN}^+ + \text{H}_2\text{O}_2 \quad (29)$$

$$\text{DPNH} + \text{H}^+ + \text{H}_2\text{O}_2 \rightarrow \text{DPN}^+ + \text{H}_2\text{O} \quad (30)$$

When DPNH is added to these extracts, 2 moles of the reduced coenzyme are oxidized per mole of O_2 reduced. The peroxidation reaction (Reaction 30) can be made limiting by dilution of the extract and under these conditions H_2O_2 accumulates.

The peroxidase which catalyzes Reaction 30 has been extensively purified and is unusual in that it contains no hematin. It does, however, contain a bound flavin, probably FAD, and small amounts of iron. The enzyme is highly resistant to azide, cyanide, and various complexing agents which chelate iron or other metals. It will not react with TPNH and is strongly inhibited by heavy metals.

In discussing the significance of the DPNH oxidase system of *S. faecalis* Dolin points out that under aerobic conditions additional sources of energy are available to the organism. Thus pyruvate, which is reduced anaerobically, can be oxidized to acetyl-CoA. DPNH, which reduces the pyruvate anaerobically in a non-phosphorylative reaction, can be oxidized through the oxidase and peroxidase system described. Since the growth of *S. faecalis* is increased 30 to 40% under aerobic conditions (*124, 253*), respiration in air can furnish energy for this increased activity. However, oxidative phosphorylation by cell-free preparations during aerobic oxidation and peroxidation has not been obtained.

Another flavoprotein enzyme which oxidizes the α-hydroxy acids, glycolic and lactic, has been observed in higher plants (*254*). This enzyme, when coupled with the reduction of glyoxylic acid by a reductase requiring either DPNH or TPNH, constitutes another pathway for oxidation of these coenzymes independent of the cytochromes (*255*). Although it has been suggested that the glycolic and glyoxylic enzymes may play a role in respiration in green leaves, there is little supporting evidence in work with intact tissues.

D. Cyanide-Insensitive Respiration

The respiration of various plant tissues is insensitive to cyanide, carbon monoxide, and azide (*227*). Enzymes other than cytochrome oxidase have therefore been suggested as the terminal oxidase in these tissues. Some results obtained with the aroid spadix, which has been studied extensively, will be described briefly.

James and Beevers (*256*) demonstrated that the respiration of tissue slices from the spadix of *Arum maculatum* was not inhibited significantly by 10^{-3} *M* HCN. However, the respiration of slices was dependent upon O_2 partial pressure and was inhibited 75% when O_2 concentration decreased from 21% to 5%. This dependence on O_2 tension and lack of inhibition by the classical inhibitors of cytochrome oxidase prompted these workers to suggest that flavoproteins mediated the reduction of O_2 in this tissue. Yocum and Hackett (*257*) in later studies confirmed that respiration of spadices of *Philodendron grandifolium* was relatively insensitive to cyanide and carbon monoxide, but they observed that the respiration rate was strongly dependent upon the manner in which O_2 was supplied to slices of this tissue. When the O_2 was furnished to the slices contained in small respirometers in a moist gas phase but with no suspending medium, the pO_2^{50} (O_2 partial pressure for half maximal respiratory rate) was 0.002 atm. However, slices in buffer exhibited a pO_2^{50} of 0.16 atm. Thus a low affinity for O_2 was observed only when the O_2 had to diffuse through the buffer to the slices.

Particulate fractions from *Peltandra virginica* spadix exhibited cytochrome oxidase activity which was inhibited by cyanide and carbon monoxide (*257*). This spadix also contained a cytochrome of the b type which appeared, from difference spectra, to function either in the presence or absence of cyanide. From these studies Yocum and Hackett concluded that cytochrome oxidase does function in the respiration of the aroid spadix. However, to account for the cyanide-insensitive respiration, Yocum and Hackett favor the view that there is an alternate, cyanide-resistant pathway of electron transfer to O_2 which involves a cytochrome of the b type. Further support for this view is found in the work of Bendall and Hill (*258*), who observed that mitochondria from spadix of *Arum maculatum* contain a cytochrome (cytochrome b_7) which does not react with CO and is oxidized by air in the presence of 10^{-3} *M* HCN.

Cytochrome c oxidase activity of mitochondria from *Arum maculatum* (*259*) and skunk cabbage (*Symplocarpus foetidus*) (*260*) spadices is inhibited strongly by cyanide (10^{-3} *M*–10^{-4} *M*). The succinoxidase activity of the same particles is also inhibited, but only to the extent of 50–60%. These results indicate that succinate oxidation can be effected by electron transfer in part through cytochrome c to O_2, and this is the amount inhibited by cyanide. However, a cyanide-insensitive route must also exist in these particles, the nature of which remains unknown. Similar conclusions result from studies on the oxidation of DPNH by skunk cabbage spadix mitochondria (*260*). Since the autoxidizable, cyanide-insensitive cytochrome b is present in these particles, it is believed to be a component. The question of whether the cyanide-resistant pathway can support oxidative phosphorylation has been raised (*257*), but no information is available on this subject.

Microsomes of both plant (*261*) and animal tissues (*262, 263*) contain cytochromes of the b type (cytochromes b_3 and b_5 respectively), and these do not appear to be affected by cyanide. However, the amount of oxidase activity in microsomes from the aroid spadix appears insufficient to account for the amount of cyanide-insensitive respiration which is present in that tissue. Since cytochrome b_3 is found in other plant tissues (*15*), its role in respiration in plants needs to be examined in more detail.

V. Conclusion

As emphasized elsewhere (*1*), half of the energy-yielding steps encountered in the metabolism of aerobic organisms occur during the transfer of electrons from the pyridine nucleotides or flavoproteins to O_2. At the many different levels of the phylogenetic scale which have

been examined, this transfer is mediated by a respiratory pathway involving cytochromes. Alternate routes for the oxidation of reduced pyridine nucleotides and flavoproteins exist, but these oxidations do not support the simultaneous formation of ATP. Similarly, organisms exist which are devoid of hematin compounds, but *oxidative* phosphorylation does not appear to be a significant energy-yielding process in those cells. It seems, therefore, that the oxidative phosphorylation process associated with the cytochrome respiratory chain may be a common denominator appearing throughout aerobic organisms. Measurements on the amount of phosphorylation associated with the cyanide-insensitive respiration of certain higher plants are not yet available.

References

1. H. A. Krebs and H. L. Kornberg, *Ergeb. Physiol* **49,** 212–298 (1957).
2. E. Bueding and E. Farber, this volume, Chapter 9.
3. Appearing in another volume in this treatise.
4. C. A. MacMunn, *Phil. Trans. Roy. Soc.* **177,** 267 (1886).
5. D. Keilin, *Proc. Roy. Soc. B***98,** 312–339 (1925).
6. D. Keilin, *Ergeb. Enzymforsch.* **2,** 239–271 (1933).
7. O. Warburg, "Heavy Metals Prosthetic Groups and Enzyme Action" (transl. A. Lawson). Oxford University Press, London and New York, 1949.
8. O. Warburg and E. Negelein, *Biochem. Z.* **214,** 64–100 (1929).
9. D. Keilin and E. F. Hartree, *Proc. Roy. Soc. B***125,** 171–186 (1938).
10. D. Keilin and E. F. Hartree, *Proc. Roy. Soc. B***127,** 167–191 (1939).
11. A. Fujita and T. Kodama, *Biochem. Z.* **273,** 186–197 (1934).
12. K.-G. Paul, *in* "The Enzymes" (J. B. Sumner and K. Myrbäck, eds.), Vol. II, Part 1, pp. 357–396. Academic Press, New York, 1951.
13. B. Chance and G. R. Williams, *Advances in Enzymol.* **17,** 65–134 (1956).
14. W. W. Wainio and S. J. Cooperstein, *Advances in Enzymol.* **17,** 329–392 (1956).
15. E. F. Hartree, *Advances in Enzymol.* **18,** 1–64 (1957).
16. L. Smith, *Bacteriol Revs.* **18,** 106–130 (1954).
17. Appearing in another volume in this treatise.
18. O. Warburg and W. Christian, *Biochem. Z.* **254,** 438–458 (1932); **266,** 377–411 (1933).
19. O. Warburg and W. Christian, *Biochem. Z.* **282,** 157–205 (1935).
20. H. Theorell, *Biochem. Z.* **288,** 317–337 (1936).
21. E. Haas, B. L. Horecker, and T. R. Hogness, *J. Biol. Chem.* **136,** 747–774 (1940).
22. B. L. Horecker, *J. Biol. Chem.* **183,** 593–605 (1950).
23. H. R. Mahler, N. K. Sarkar, L. P. Vernon, and R. A. Alberty, *J. Biol. Chem.* **199,** 585–597 (1952).
24. P. Strittmatter and S. F. Velick, *J. Biol. Chem.* **228,** 785–799 (1957).
25. H. R. Mahler, *Advances in Enzymol.* **17,** 233–291 (1956).
26. H. Theorell, *in* "The Enzymes" (J. B. Sumner and K. Myrbäck, eds.), Vol. II, Part 1, pp. 335–356. Academic Press, New York, 1951.
27. O. Warburg and W. Christian, *Biochem. Z.* **242,** 206–227 (1931).
28. A. Harden and W. J. Young, *Proc. Roy. Soc. B***77,** 405–420 (1906).

29. F. Schlenk, *in* "The Enzymes" (J. B. Sumner and K. Myrbäck, eds.), Vol. II, Part 1, pp. 250–315. Academic Press, New York, 1951.
30. T. P. Singer and E. B. Kearney, *Advances in Enzymol.* **15,** 79–139 (1954).
31. B. Vennesland, *J. Cellular Comp. Physiol.* **47,** Suppl. 1, 201–216 (1956).
32. R. Kuhn and P. Boulanger, *Ber.* **69,** 1557–1566 (1936).
33. T. E. Coolidge, *J. Biol. Chem.* **98,** 755–764 (1932).
34. E. G. Ball and P. A. Ramsdell, *J. Biol. Chem.* **131,** 767–768 (1939).
35. E. G. Ball, *Biochem. Z.* **295,** 262–264 (1938).
36. D. Keilin and E. F. Hartree, *Proc. Roy. Soc.* **B129,** 277–306 (1940).
37. E. G. Ball, *Cold Spring Harbour Symposia Quant. Biol.* **7,** 100–108 (1939).
38. H. A. Neufeld, C. R. Scott, and E. Stotz, *J. Biol. Chem.* **210,** 869–876 (1954).
39. T. P. Singer, E. B. Kearney, and V. Massey, *Advances in Enzymol.* **18,** 65–111 (1957).
40. D. E. Green, S. Mii, and P. M. Kohout, *J. Biol. Chem.* **217,** 551–567 (1955).
41. E. C. Slater, *Biochem. J.* **46,** 484–499 (1950).
42. E. C. Slater, *Biochem. J.* **45,** 14–30 (1949).
43. A. E. Reif and V. R. Potter, *Arch. Biochem. Biophys.* **48,** 1–6 (1954).
44. L. Ernster, O. Jalling, H. Löw, and O. Lindberg, *Exptl. Cell Research* Suppl. 3, 124–132 (1955).
45. H. W. Clark, H. A. Neufeld, C. Widmer, and E. Stotz, *J. Biol. Chem.* **210,** 851–859 (1954).
46. H. R. Mahler, *Ann. Rev. Biochem.* **26,** 17–62 (1957).
47. B. Chance, *in* "Enzymes: Units of Biological Structure and Function" (O. H. Gaebler, ed.), pp. 447–463. Academic Press, New York, 1956.
48. E. C. Slater, *Ann. Rev. Biochem.* **22,** 17–56 (1953).
49. T. M. Devlin and A. L. Lehninger, *J. Biol. Chem.* **219,** 507–518 (1956).
50. G. F. Maley, *J. Biol. Chem.* **224,** 1029–1038 (1957).
51. B. Chance, *Nature* **169,** 215–221 (1952).
52. B. Chance and G. R. Williams, *Nature* **176,** 250–254 (1955).
53. F. L. Crane, J. L. Glenn, and D. E. Green, *Biochim. et Biophys. Acta* **22,** 475–487 (1956).
54. B. Mackler and D. E. Green, *Biochim. et Biophys. Acta* **21,** 6–13 (1956).
55. D. E. Green, *in* "Enzymes: Units of Biological Structure and Function" (O. H. Gaebler, ed.), pp. 465–481. Academic Press, New York, 1956.
56. F. L. Crane and J. L. Glenn, *Biochim. et Biophys. Acta* **24,** 100–107 (1957).
57. R. E. Basford, H. D. Tisdale, J. L. Glenn, and D. E. Green, *Biochim. et Biophys. Acta* **24,** 107–115 (1957).
58. B. Mackler and D. E. Green, *Biochim. et Biophys. Acta* **24,** 100–107 (1957).
59. B. Mackler and N. Penn, *Biochim. et Biophys. Acta* **24,** 294–300 (1957).
60. J. L. Gamble, Jr., and A. L. Lehninger, *J. Biol. Chem.* **223,** 921–933 (1956).
61. A. Claude, *Harvey Lectures* **43,** 121–164 (1949).
62. G. H. Hogeboom, W. C. Schneider, and M. J. Striebich, *Cancer Research* **13,** 617–632 (1953).
63. W. C. Schneider, *J. Biol. Chem.* **165,** 585–593 (1946).
64. G. H. Hogeboom, A. Claude, and D. Hotchkiss, *J. Biol. Chem.* **165,** 615–629 (1946).
65. B. Chance, *Science* **120,** 767–775 (1954).
66. B. Chance and G. R. Williams, *J. Biol. Chem.* **217,** 409–427 (1955).
67. B. Chance and G. R. Williams, *J. Biol. Chem.* **217,** 383–393 (1955).
68. B. Chance and G. R. Williams, *J. Biol. Chem.* **217,** 429–451 (1955).

69. B. Chance and G. R. Williams, *J. Biol. Chem.* **217,** 395–407 (1955).
70. B. Chance, *in* "Mechanism of Enzyme Action" (W. D. McElroy and B. Glass, eds.), pp. 399–453. Johns Hopkins, Baltimore, 1954.
71. B. Chance, *Trans. N. Y. Acad. Sci.* **16,** 74–75 (1953).
72. T. P. Singer and E. B. Kearney, *J. Biol. Chem.* **223,** 599–613 (1956).
73. R. E. Basford, H. D. Tisdale, and D. E. Green, *Biochim. et Biophys. Acta* **24,** 290–300 (1957).
74. E. H. Stotz, M. Morrison, and G. Marinetti, *in* "Enzymes: Units of Biological Structure and Function" (O. H. Gaebler, ed.), pp. 401–416. Academic Press, New York, 1956.
75. C. Cooper and A. L. Lehninger, *J. Biol. Chem.* **219,** 489–506 (1956).
76. C. Cooper and A. L. Lehninger, *J. Biol. Chem.* **219,** 519–529 (1956).
77. D. M. Ziegler, R. L. Lester, and D. E. Green, *Biochim. et Biophys. Acta* **21,** 80–91 (1956).
78. D. E. Green, R. L. Lester, and D. M. Ziegler, *Biochim. et Biophys. Acta* **23,** 516–524 (1957).
79. W. C. McMurray, G. F. Maley, and H. A. Lardy, *J. Biol. Chem.* **230,** 219–229 (1958).
80. W. W. Kelley and J. R. Bronk, *J. Biol. Chem.* **230,** 521–533 (1958).
81. H. Lundegardh, *Nature* **171,** 521–522 (1953).
82. H. Lundegardh, *Biochim. et Biophys. Acta* **20,** 469–487 (1956).
83. A. L. Lehninger, *J. Biol. Chem.* **190,** 345–359 (1951).
84. V. R. Potter, *J. Biol. Chem.* **141,** 775–787 (1941).
85. W. C. Schneider and V. R. Potter, *J. Biol. Chem.* **149,** 217–227 (1943).
86. D. Keilin and E. F. Hartree, *Biochem. J.* **41,** 500–502 (1951).
87. E. G. Ball and O. Cooper, *J. Biol. Chem.* **180,** 113–124 (1949).
88. A. P. Nygaard, *J. Biol. Chem.* **204,** 655–663 (1953).
89. F. M. Huennekens, R. E. Basford, and B. W. Gabrio, *J. Biol. Chem.* **213,** 951–967 (1955).
90. I. R. Lehman and A. Nason, *J. Biol. Chem.* **222,** 497–509 (1956).
91. B. Sacktor, *Arch. Biochem. Biophys.* **45,** 349–365 (1953).
92. A. M. Pappenheimer, Jr., and C. M. Williams, *J. Biol. Chem.* **209,** 915–929 (1954).
93. M. I. Watanabe and C. M. Williams, *J. Gen. Physiol.* **34,** 675–689 (1950–1951).
94. H. J. Eichel, *J. Biol. Chem.* **206,** 159–169 (1954).
95. H. J. Eichel, *J. Biol. Chem.* **222,** 121–136 (1956).
96. K. Bhagvat and R. Hill, *New Phytologist* **50,** 112–120 (1951).
97. H. A. Stafford, *Physiol. Plantarum* **4,** 696–741 (1951).
98. A. Millerd, *Proc. Linnean Soc. N. S. Wales* **76,** 123 (1951).
99. E. R. Waygood, *Can. J. Research* **C28,** 7–62 (1950).
100. H. T. Freebairn and L. F. Remmert, *Plant Physiol.* **31,** 259–266 (1956).
101. D. P. Hackett, *Intern. Rev. Cytol.* **4,** 143–196 (1955).
102. D. P. Hackett, *Plant Physiol.* **31,** 111–118 (1956).
103. T. E. Humphreys and E. E. Conn, *Arch. Biochem. Biophys.* **60,** 226–243 (1956).
103a. D. P. Hackett, *J. Exptl. Botany* **8,** 157–171 (1957).
104. L. P. Vernon, H. R. Mahler, and N. K. Sarkar, *J. Biol. Chem.* **199,** 599–606 (1952).
105. A. W. Linnane and J. L. Still, *Arch. Biochem. Biophys.* **59,** 383–391 (1955).

106. T. P. Singer, V. Massey, and E. B. Kearney, *Arch. Biochem. Biophys.* **69,** 405–421 (1957).
107. A. Tissieres, H. G. Hovenkamp, and E. C. Slater, *Biochim. et Biophys. Acta* **25,** 336–347 (1957).
108. M. Alexander and P. W. Wilson, *Proc. Natl. Acad. Sci. U. S.* **41,** 843–848 (1955).
109. J. H. Bruemmer, P. W. Wilson, J. L. Glenn, and F. L. Crane, *J. Bacteriol.* **73,** 113–116 (1951).
110. I. A. Rose and S. Ochoa, *J. Biol. Chem.* **220,** 307–314 (1956).
111. A. W. Linnane and J. L. Still, *Biochim. et Biophys. Acta* **16,** 305–306 (1955).
112. A. F. Brodie and C. T. Gray, *J. Biol. Chem.* **219,** 853–862 (1956).
113. A. F. Brodie and C. T. Gray, *Biochim. et Biophys. Acta* **19,** 384–386 (1956).
114. D. Billen and E. Volkin, *J. Bacteriol.* **67,** 191–197 (1954).
115. A. Tissieres, *Nature* **169,** 880–881 (1952).
116. L. Burns and W. Militzer, *Proc. Soc. Exptl. Biol. Med.* **82,** 411–413 (1953).
117. M. D. Kamen and L. P. Vernon, *J. Bacteriol.* **67,** 617–618 (1954).
118. J. R. Postgate, *Biochim. et Biophys. Acta* **18,** 427–428 (1955).
119. J. W. Newton and M. D. Kamen, *Arch. Biochem. Biophys.* **58,** 246–247 (1955).
120. M. D. Kamen and L. P. Vernon, *Biochim. et Biophys. Acta* **17,** 10–22 (1955).
121. L. P. Vernon, *J. Biol. Chem.* **222,** 1035–1044 (1956).
122. M. I. Dolin, *Arch. Biochem. Biophys.* **46,** 483–485 (1953).
123. L. P. Vernon and M. D. Kamen, *J. Biol. Chem.* **211,** 643–662 (1954).
124. M. I. Dolin, *Arch. Biochem. Biophys.* **55,** 415–435 (1955).
125. D. Keilin and A. Tissieres, *Nature* **172,** 393–394 (1953).
126. J. B. Nielands, *J. Biol. Chem.* **197,** 701–708 (1952).
127. R. T. Darby and D. R. Goddard, *Physiol. Plantarum* **3,** 435–446 (1950).
128. H. Tamiya, *Advances in Enzymol.* **2,** 183–238 (1942).
129. D. Boulter and E. Derbyshire, *J. Exptl. Botany* **8,** 313–318 (1957).
130. H. Lundegardh, *Ann. Rev. Plant Physiol.* **6,** 1–24 (1955).
131. R. Hill and E. F. Hartree, *Ann. Rev. Plant Physiol.* **4,** 115–150 (1953).
132. R. H. Nieman and B. Vennesland, *Science* **125,** 353–354 (1957).
133. H. Kalckar, *Enzymologia* **2,** 47–52 (1937).
134. V. A. Belitzer and E. T. Tsibakowa, *Biokhimiya* **4,** 516–535 (1939).
135. S. Ochoa, *J. Biol. Chem.* **151,** 493–505 (1943).
136. F. E. Hunter, Jr., *in* "Phosphorus Metabolism" (W. D. McElroy and B. Glass, eds.), Vol. I, pp. 297–330. Johns Hopkins, Baltimore, 1951.
137. A. L. Lehninger, *Harvey Lectures* **49,** 176–215 (1955).
137a. *See* F. M. Huennekins and H. R. Whiteley, this treatise, Vol. I, Chapter 4; *also* M. R. Atkinson and R. K. Morton, Vol. II, Chapter 1.
138. V. A. Belitzer, *Enzymologia* **6,** 1–8 (1939).
139. S. P. Colowick, M. Welch, and C. F. Cori, *J. Biol. Chem.* **133,** 359–373, 641–642 (1940).
140. S. Ochoa, *J. Biol. Chem.* **138,** 751–772 (1941).
141. R. Cross, J. Taggart, G. Covo, and D. E. Green, *J. Biol. Chem.* **177,** 655–678 (1949).
142. M. Friedkin and A. L. Lehninger, *J. Biol. Chem.* **177,** 775–788 (1949).
143. F. E. Hunter, Jr., *J. Biol. Chem.* **177,** 361–372 (1949).
144. A. L. Lehninger and E. P. Kennedy, *J. Biol. Chem.* **173,** 753–770 (1948).

145. A. L. Lehninger, *J. Biol. Chem.* **178,** 625–644 (1949).
146. J. H. Copenhaver and H. A. Lardy, *J. Biol. Chem.* **195,** 225–238 (1952).
147. W. W. Kielley and R. K. Kielley, *J. Biol. Chem.* **191,** 485–500 (1951).
148. G. F. Maley and G. W. E. Plaut, *J. Biol. Chem.* **205,** 297–302 (1953).
149. F. E. Hunter, Jr., and W. S. Hixson, *J. Biol. Chem.* **181,** 73–79 (1949).
150. B. R. Johnson and H. A. Lardy, *J. Biol. Chem.* **184,** 235–242 (1950).
151. E. C. Slater, *Biochem. J.* **53,** 521–530 (1953).
152. E. C. Slater, *Biochem. J.* **53,** 157–167 (1953).
153. S. O. Nielsen and A. L. Lehninger, *J. Biol. Chem.* **215,** 555–570 (1955).
154. H. A. Krebs, A. Ruffo, M. Johnson, L. V. Eggleston, and R. Hems, *Biochem. J.* **54,** 107–116 (1953).
155. E. C. Slater and F. A. Holton, *Biochem. J.* **56,** 28–40 (1954).
156. S. Barkulis and A. L. Lehninger, *J. Biol. Chem.* **193,** 597–604 (1951).
157. M. Friedkin and A. L. Lehninger, *J. Biol. Chem.* **178,** 611–623 (1949).
158. A. L. Lehninger, *J. Biol. Chem.* **190,** 345–359 (1951).
159. A. L. Lehninger, *in* "Symposium on Phosphorus Metabolism" (W. D. McElroy and B. Glass, eds.), Vol. I, pp. 344–366. Johns Hopkins, Baltimore, 1951.
160. G. F. Maley, *J. Biol. Chem.* **224,** 1029–1038 (1957).
161. N. O. Kaplan, M. N. Swartz, M. E. Frech, and M. M. Ciotti, *Proc. Natl. Acad. Sci. U. S.* **42,** 481–487 (1956).
162. N. O. Kaplan, S. P. Colowick, and E. F. Neufeld, *J. Biol. Chem.* **205,** 1–15 (1953).
163. P. V. Vignais and P. M. Vignais, *J. Biol. Chem.* **229,** 265–278 (1957).
164. J. D. Judah, *Biochem. J.* **49,** 271–285 (1951).
165. G. F. Maley and H. A. Lardy, *J. Biol. Chem.* **210,** 903–909 (1954).
166. A. L. Lehninger, M. Ul Hassan, and H. C. Sudduth, *J. Biol. Chem.* **210,** 911–922 (1954).
167. E. C. Slater, *Nature* **174,** 1143–1144 (1954).
168. E. C. Slater, *Nature* **166,** 982–984 (1950).
168a. B. Borgström, H. C. Sudduth, and A. L. Lehninger, *J. Biol. Chem.* **215,** 571 (1955).
169. B. Chance, G. R. Williams, W. F. Holmes, and J. Higgins, *J. Biol. Chem.* **217,** 439–451 (1955).
170. E. C. Slater, *Nature* **172,** 975–978 (1953).
171. F. Lipmann, *in* "Currents in Biochemical Research" (D. E. Green, ed.), pp. 137–148. Interscience, New York, 1946.
172. D. K. Myers and E. C. Slater, *Nature* **179,** 363–364 (1957).
173. W. C. Hulsmann and E. C. Slater, *Nature* **180,** 372–374 (1957).
174. C. Cooper and A. L. Lehninger, *J. Biol. Chem.* **224,** 561–578 (1957).
175. B. Chance and G. R. Williams, *Nature* **176,** 250–254 (1955).
176. E. Racker, *in* "Mechanism of Enzyme Action" (W. D. McElroy and B. Glass, eds.), pp. 464–490. Johns Hopkins, Baltimore, 1954.
177. C. L. Wadkins and A. L. Lehninger, *J. Am. Chem. Soc.* **79,** 1010–1011 (1957).
178. B. Chance and G. R. Williams, *J. Biol. Chem.* **221,** 477–489 (1956).
179. M. Cohn and G. R. Drysdale, *J. Biol. Chem.* **216,** 831–846 (1955).
180. D. R. Sanadi, D. M. Gibson, P. Ayengar, and M. Jacob, *J. Biol. Chem.* **218,** 505–520 (1956).
181. B. Chance and G. R. Williams, *Nature* **176,** 250–254 (1955).

182. W. F. Loomis and F. Lipmann, *J. Biol. Chem.* **173,** 807–808 (1948).
183. H. A. Lardy and H. Wellman, *J. Biol. Chem.* **201,** 357–370 (1953).
184. C. Cooper and A. L. Lehninger, *J. Biol. Chem.* **224,** 547–578 (1957).
185. A. L. Lehninger, *in* "Enzymes: Units of Biological Structure and Function (O. H. Gaebler, ed.), pp. 217–233. Academic Press, New York, 1956.
186. L. Ernster and O. Lindberg, *Acta Chem. Scand.* **8,** 1096 (1955).
187. D. F. Tapley, *J. Biol. Chem.* **222,** 325–339 (1956).
188. D. F. Tapley and C. Cooper, *J. Biol. Chem.* **222,** 341–349 (1956).
189. H. E. Robertson and P. D. Boyer, *J. Biol. Chem.* **214,** 295–305 (1955).
190. P. D. Boyer, A. B. Falcone, and W. H. Harrison, *Nature* **174,** 401–40 (1954).
191. K. W. Cleland and E. C. Slater, *Biochem. J.* **53,** 547–556 (1953).
192. G. F. Maley and H. A. Lardy, *J. Biol. Chem.* **204,** 435–444 (1953).
193. M. Dianzani, *Biochim. et Biophys. Acta* **14,** 514–532 (1954).
194. L. G. Abood and L. Alexander, *J. Biol. Chem.* **227,** 717–725 (1957).
195. W. Bartley, *Biochem. J.* **54,** 677–682 (1953).
196. M. Rabinowitz and P. D. Boyer, *Proc. Soc. Exptl. Biol. Med.* **77,** 103–10 (1951).
197. S. H. Mudd, J. H. Park, and F. Lipmann, *Proc. Natl. Acad. Sci. U. S.* **4** 571–576 (1955).
198. E. C. Weinbach, *Arch. Biochem. Biophys.* **64,** 129–143 (1956).
199. K. R. Rees, *Biochem. J.* **58,** 196–202 (1954).
200. S. E. Lewis and E. C. Slater, *Arch. Biochem. Biophys.* **58,** 207–217 (1954).
201. B. Sacktor, *J. Gen. Physiol.* **37,** 343–359 (1954).
202. O. Gonda, A. Traub, and Y. Avi-dor, *Biochem. J.* **67,** 487–493 (1957).
203. G. Chin and E. Bueding, *Biochim. et Biophys. Acta* **13,** 331–337 (1954).
204. A. Millerd, J. Bonner, B. Axelrod, and R. Bandurski, *Proc. Natl. Acad. Sc U. S.* **37,** 855–862 (1951).
205. A. Millerd and J. Bonner, *Arch. Biochem. Biophys.* **42,** 149–152 (1953).
206. G. G. Laties, *Plant Physiol.* **28,** 557–575 (1953).
207. J. B. Biale, R. E. Young, C. S. Popper, and W. E. Appleman, *Physi Plantarum* **10,** 48–63 (1957).
208. E. E. Conn and L. C. T. Young, *J. Biol. Chem.* **226,** 23–32 (1957).
209. M. Lieberman and J. B. Biale, *Plant Physiol.* **31,** 420–424 (1956).
210. H. T. Freebairn and L. F. Remmert, *Plant Physiol.* **32,** 374–376 (1957).
211. R. M. Smillie, *Australian J. Biol. Sci.* **8,** 186–195 (1955).
212. R. M. Smillie, *Australian J. Biol. Sci.* **9,** 81–91 (1956).
213. C. M. Switzer, *Plant Physiol.* **32,** 42–44 (1957).
214. C. M. Switzer and F. G. Smith, *Can. J. Botany* **35,** 515–525 (1957).
215. T. Akazawa and H. Beevers, *Biochem. J.* **67,** 113–118 (1957).
216. T. Ohmura, *Arch. Biochem. Biophys.* **57,** 187–194 (1955).
217. R. M. Smillie, *Australian J. Biol. Sci.* **9,** 347–354 (1956).
218. B. A. Bonner and L. Machlis, *Plant Physiol.* **32,** 291–301 (1957).
219. L. A. Hyndman, R. H. Burris, and P. W. Wilson, *J. Bacteriol.* **65,** 522–5 (1953).
220. G. B. Pinchot, *J. Biol. Chem.* **205,** 65–74 (1953).
221. G. B. Pinchot, *J. Biol. Chem.* **229,** 1–9 (1957).
222. G. B. Pinchot, *J. Biol. Chem.* **229,** 25–37 (1957).
223. G. B. Pinchot, *J. Biol. Chem.* **229,** 11–23 (1957).
224. W. D. Bonner, Jr., *Ann. Rev. Plant Physiol.* **8,** 427–452 (1957).

225. D. R. Goddard and B. J. D. Meeuse, *Ann. Rev. Plant Physiol.* **1,** 207–232 (1950).
226. R. Hill and E. F. Hartree, *Ann. Rev. Plant Physiol.* **4,** 115–150 (1953).
227. W. O. James, "Plant Respiration." Oxford University Press, London and New York, 1953.
228. D. P. Hackett and H. A. Schneiderman, *Arch. Biochem. Biophys.* **47,** 190–204 (1953).
229. K. V. Thimann, C. S. Yocum, and D. P. Hackett, *Arch. Biochem. Biophys.* **53,** 239–257 (1954).
230. L. Ordin and L. Jacobson, *Plant Physiol.* **30,** 21–27 (1955).
231. C. G. Laties, *Survey Biol. Progr.* **3,** 215–299 (1957).
232. H. S. Mason, *Advances in Enzymol.* **19,** 79–233 (1957).
233. F. J. Dunn and C. R. Dawson, *J. Biol. Chem.* **189,** 485–497 (1951).
234. W. O. James, *Advances in Enzymol.* **18,** 281–318 (1957).
235. L. W. Mapson and E. M. Moustafa, *Biochem. J.* **62,** 248–259 (1956).
236. B. Vennesland and E. E. Conn, in "Glutathione, A Symposium" (S. P. Colowick *et al.*, eds.), pp. 105–126. Academic Press, New York, 1954.
237. L. W. Mapson, *Vitamins and Hormones* **11,** 1–28 (1953).
238. M. Calvin, in "Glutathione, A Symposium" (S. P. Colowick *et al.*, eds.), pp. 3–26. Academic Press, New York, 1954.
239. L. C. T. Young and E. E. Conn, *Plant Physiol.* **31,** 205–211 (1956).
240. R. S. Russell, *Symposia Soc. Exptl. Biol.* **8,** 343–366 (1954).
241. M. B. Mathews, *J. Biol. Chem.* **189,** 695–704 (1951).
242. A. Nason, W. D. Wosilait, and A. J. Terrell, *Arch. Biochem. Biophys.* **48,** 233–235 (1954).
243. H. Beevers, *Plant Physiol.* **29,** 265–269 (1954).
244. M. Kern and E. Racker, *Arch. Biochem. Biophys.* **48,** 235–236 (1954).
245. C. R. Dawson and W. B. Tarpley, in "The Enzymes" (J. B. Sumner and K. Myrbäck, eds.), Vol. II, Part 1, pp. 454–498. Academic Press, New York, 1951.
246. H. S. Mason, *Advances in Enzymol.* **16,** 105–184 (1955).
247. F. Kubowitz, *Biochem. Z.* **299,** 32–57 (1938).
248. W. D. Wosilait and A. Nason, *J. Biol. Chem.* **206,** 255–270 (1954).
249. W. D. Wosilait, A. Nason, and A. J. Terrell, *J. Biol. Chem.* **206,** 271–282 (1954).
250. D. R. Goddard and C. Holden, *Arch. Biochem.* **27,** 41–47 (1950).
251. M. I. Dolin, *Arch. Biochem. Biophys.* **60,** 499–501 (1956).
252. M. I. Dolin, *J. Biol. Chem.* **225,** 557–573 (1957).
253. H. W. Seeley and P. J. Vandemark, *J. Bacteriol.* **61,** 27–35 (1951).
254. I. Zelitch and S. Ochoa, *J. Biol. Chem.* **201,** 707–718 (1953).
255. I. Zelitch, *J. Biol. Chem.* **201,** 719–726 (1953).
256. W. O. James and H. Beevers, *New Phytologist* **49,** 353–374 (1950).
257. C. S. Yocum and D. P. Hackett, *Plant Physiol.* **32,** 186–191 (1957).
258. D. S. Bendall and R. Hill, *New Phytologist* **55,** 206–212 (1956).
259. E. W. Simon, *J. Exptl. Botany* **8,** 20–35 (1957).
260. D. P. Hackett, *J. Exptl. Botany* **8,** 157–171 (1957).
261. E. M. Martin and R. K. Morton, *Biochem. J.* **62,** 696–704 (1956).
262. B. Chance and G. R. Williams, *J. Biol. Chem.* **209,** 945–951 (1954).
263. P. Strittmatter and S. F. Velick, *J. Biol. Chem.* **221,** 253–264 (1956).

CHAPTER 11

Utilization of Thermal Energy by Living Organisms

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I. The Need of All Organisms for Thermal Energy: General Considerations

The life of all organisms depends upon the maintenance of an adequate supply of thermal energy. This energy is partly received from the environment and partly generated by the organisms themselves during their metabolism. Living creatures require thermal energy to maintain their cellular constituents in a physical state which makes metabolism possible and to supply the activation energy needed for vital reactions. Although enzymes are known which will continue to react in the frozen state (*1–3*), no organism has been found which can carry on its life processes when the cellular contents are frozen. Enzyme catalysis may reduce, but does not eliminate, the need for activation energy to carry out the chemical reactions necessary for life and growth. At the same time, however, thermal energy is a destructive force which tends to break down the delicate mechanisms of the cell. Metabolic work must be expended to repair enzymes and other cellular constituents which have been disrupted by thermal effects. For some organisms living at

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high temperatures, the ability to repair metabolically the damaging effects of heat may mark the difference between life and death (*4, 5*).

Living organisms have been able to balance these two contrasting effects so that life in some form occurs within the whole range in which water, that most important of all biocatalysts, is fluid. The biokinetic zone is bounded on its lower limit by the freezing point of the cell contents and surrounding medium (which may be well below 0° in media of high osmotic concentration), and on its upper limit by the boiling point of water. For most individual organisms, however, the zone of permissible temperatures is much narrower. Many forms of life die of cold at temperatures well above freezing, and only a few specially adapted organisms can endure temperatures above 50–55°, where many of the usual enzymatic proteins are irreversibly damaged by heat. Temperature limits for the vital processes of a variety of organisms are given in Table I.

It may be desirable at this point to define some of the terms which are used in speaking of the relation of organisms to temperature. Those which can survive and develop over a wide temperature range are referred to as eurythermal, whereas those which are limited to a narrow temperature band are called stenothermal. Organisms which develop at low temperatures are known as cryophilic; those which prefer moderate temperatures as mesophilic; and those which can withstand or which require high temperatures for growth as thermophilic. The temperatures used to delimit these zones vary with the type of organism being discussed. For example, bacteria are not usually considered thermophilic unless they can grow at 55° or above, whereas the term is applied to green algae which have an optimum temperature at 40° and a maximum only slightly higher. The thermophilic organisms which have attracted the greatest biochemical interest are those which can survive above the coagulation temperature of many usual enzyme proteins, and which are often unable to grow at more usual temperatures.

Those animals, including the birds and mammals, which have learned to use metabolic energy to maintain a more or less constant internal thermic environment are known as homoiotherms, whereas all the organisms whose internal temperature reflects the temperature of their surroundings are known as poikilotherms.

Increases in temperature generally tend to increase the rate of chemical reactions, whether these occur within or without the living organism. In living creatures, however, influences of competing reactions, hormonal control, etc. may act in such a way that the observed over-all response to temperature of complex processes, e.g., growth and energy production, is different from that predicted by physicochemical theory,

even though the individual reactions taking place in the organism are governed by the same rules which control them *in vitro*. These rules, however, remain of importance when a molecular explanation of biochemical events is sought.

TABLE I
REPRESENTATIVE TEMPERATURE LIMITS FOR GROWTH AND DEVELOPMENT

Organism	Minimum Temperature (° C)	Maximum Temperature (° C)
Alligator (*Alligator mississippiensis*)	4	39
European lizard (*Lacerta agilis*)	−4	44
Garter snake (*Thamnophis radix*)	0	41
Painted turtle (*Pseudemys elegans*)	1	46
Green frog (*Rana clamitans*)	−0.5	22
Common toad (*Bufo bufo*)	−1	33
Western pine beetle (*Dendroctonus varivestis*)	4–7	37.8–40.6
Boll weevil (*Anthonomus grandis*)	−4 to 3	50–60
Housefly (*Musca domestica*)	6.7–7.2	44.6
Bedbug (*Cimex lectularis*)	7.5	34–43.5
Ancylostoma caninum (Nematode)	15	37
Trichuris trichiura (Nematode)	−9 to −12	54
Achromobacter ichthyodermis	−2	30
Corynebacterium diphtheriae	15	40
Clostridium botulinum	18	55
Bacillus subtilis	15	55
Stenothermal thermophils	35	75–85
Myxophyta[a]	−17	85
Rhodophyta[a]	−20	35
Chlorophyta[a]	−20	55
Phaeophyta[a]	−20	35
Arctic fir	−40 (Survives at −60°)	30
Date palm	10	54

[a] No single species has the wide limits shown here. The figures indicate the range possible for the group. [Data from W. S. Spector, ed., "Handbook of Biological Data." National Academy of Sciences–National Research Council, Washington, D. C., 1956.]

The formulation of temperature effects on reaction rates which has been found most generally applicable to biochemical systems is the statistical mechanical treatment developed by Eyring, Stearns, and their collaborators. This subject has been treated extensively in this treatise and elsewhere (see *5a–9*).

However, for many qualitative and semiquantitative purposes, particularly when it is desired to compare one organism or reacting system with another over a limited temperature range, a simple linear temperature coefficient suffices. This is usually expressed as Q_{10}, the increase in reaction rate caused by a 10° rise in temperature. When a wide temperature interval is investigated, Q_{10} usually varies with the temperature, since the actual temperature-rate relationship is logarithmic rather than linear.

II. Methods of Meeting Thermal Requirements

A. Poikilotherms

Although all organisms generate heat in the course of their metabolism, the lower organisms do not have a mechanism for using this metabolic energy to maintain a constant internal thermic environment. Although larger forms may become temporarily warmer than their surroundings during periods of activity, small organisms have too great a heat loss to maintain a temperature appreciably different from that of their surroundings (with certain exceptions, which are discussed below). In all cases, however, these poikilothermic organisms are dependent on an environment which will provide a temperature favorable for their life processes. Under natural conditions, this usually means that their metabolism must cope with a great deal of diurnal and seasonal temperature variation. This is sometimes reflected by alterations in the optimum temperature and temperature coefficients of enzyme activity. The biochemical basis of these alterations is little understood. Some organisms, notably the higher plants, have even become dependent on regular temperature differences between day and night for the proper completion of their development cycle (*10*, *11*). Here again, the biochemical reactions underlying this need for a temperature cycle are practically unknown. (Discussion of the temperature cycles of higher plants is considered to be outside the scope of this review. For exposition of this subject see Went, *11*).

The primary external source of thermal energy for most organisms is sunlight. (The exceptions are warm springs and other environments which contain an alternative source of heat.) The total solar radiation reaching the earth is approximately 2 cal./minute/cm.^{2} (*12*). The fate of this solar energy is shown diagrammatically in Fig. 1. For organisms at the surface of the soil both direct and reflected sunlight contribute to the thermal energy available.

The extent to which this radiation raises the temperature of an organism depends upon its thermal conductivity. Thin leaves rapidly

reach a state of thermal equilibrium with the atmosphere, whereas fleshy organisms do so much more slowly. Coatings which act as insulators may influence the result. Penetration of infrared radiation into organisms has been reported to be able to raise their temperature as much as 20–30° above that of the surrounding atmosphere (*13–15*). This excess thermal energy tends to be lost through convection, reradiation,

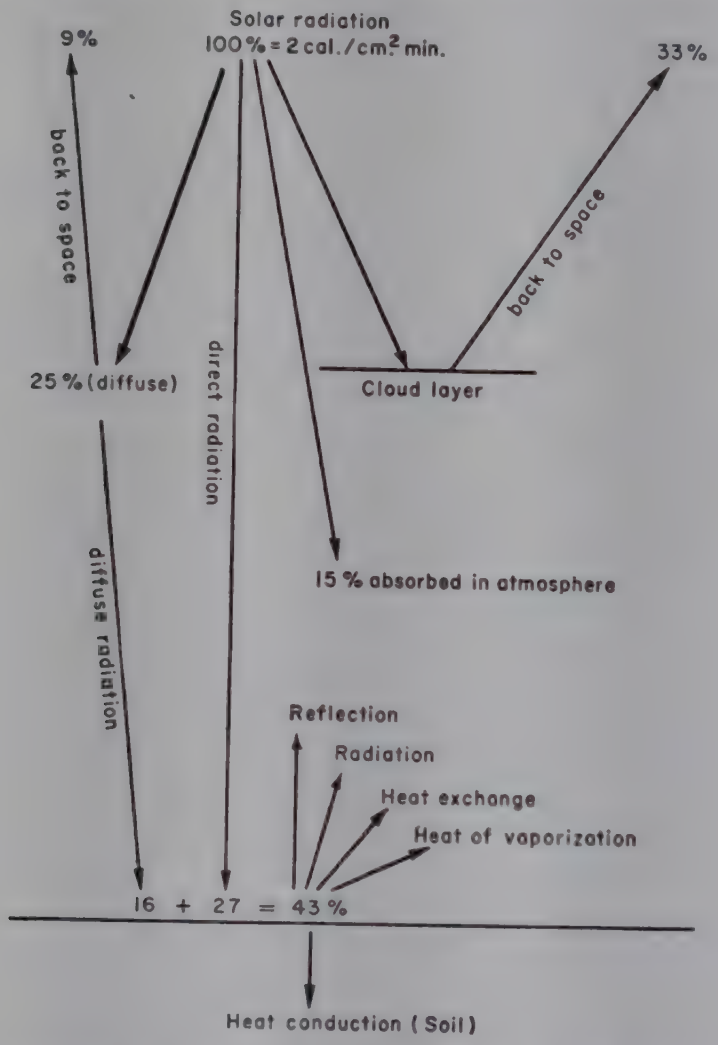


FIG. 1. Heat exchange at the earth's surface and in the atmosphere at midday, according to H. Walter ["Einführung in die Phytologie, III: Grundlagen der Pflanzenverbreitung." E. Ulmen, Stuttgart, 1951]. The numbers represent average values.

and evaporation of water. The relative importance of these modes of heat loss varies for different organisms (*16, 17*). Color has a marked effect on the extent to which organisms are warmed by sunlight. Members of dark-colored varieties reach higher temperatures than those which are light in color (*18–21*) and hence tend to predominate in environments, e.g., high mountains and arctic regions, where temperature is often a limiting factor in development. Organisms with chromatophores which can be expanded and contracted may show a darkening at low temperatures and a bleaching at high ones (*22*).

Since plants are immobile, they are especially inclined to temperature changes in sunlight. Photosynthesis utilizes only a portion of the energy absorbed by a green leaf; the remainder goes to raise its temperature. In shade, on the other hand, evaporation of water may cool leaves below the temperature of their surroundings. Roots generally follow the temperature of the soil around them. Succulents, because of their large ratio of volume to surface, are particularly prone to being warmed above the temperature of their surroudings by sunlight. Excess temperatures of as much as 30° have been reported (*23, 24*).

Internal heat due to metabolism, although generally less than that produced by homoiothermic organisms, is also of importance to poikilotherms. The ratio of surface to volume is of considerable importance in determining the contribution which metabolic energy makes to the thermal economy of the organism. Large creatures, e.g. tuna fish and pythons, can reach temperatures as much as 10° above their surroundings as the result of active metabolism (*25, 26, 27*), whereas smaller forms rapidly lose their metabolic heat through exchange with the environment. However, insects which are individually too small to retain much metabolic heat may do so by congregating in swarms (*28*). Intensive muscular activity may also result in raising even small organisms, flying insects for example, above the temperature of their surroundings (*15, 29*). Among plants, rapidly metabolizing tissues such as palm shoots, lily bulbs, and parts of cycads have been observed to reach temperatures 10–20° above that of the surrounding environment (*30, 31, 32*).

Organisms lose thermal energy to the environment by a number of processes. These include: (*a*) conduction, which is of greater importance to aquatic forms than to terrestrial ones, since water is a much better conductor of heat than is air; (*b*) convection, which is involved in processes such as transport of heat to the body surface by circulating blood, and loss of thermal energy from the surface to moving air; (*c*) radiation, which for a black body is given by the equation

$$S = 5.75 \times 10^{-9} T^4 \text{ mw./cm.}^2$$

Since the radiation emitted by bodies in the biological temperature range is of very long wavelength ($\sim 10\ \mu$), the visible color of an organism will have little effect on its ability to radiate (*12*).

Evaporation of water plays an important role. The extent of its contribution depends on the relative humidity of the surrounding environment and on whether the air is still or moving. In dry air, insects (*33–35*) and plants (*30, 36*) have been observed to maintain temperatures materially below those of the surrounding atmosphere as a result of evaporative cooling. Thin skinned amphibians rely to a great extent

on evaporation of water to prevent overheating. Those which cannot evaporate water freely, due to scaly or horny skins, must avoid the heating effects of direct sunlight (cf. reference *37*).

It must not be concluded that because the poikilotherms have little or no ability to regulate their internal temperatures their metabolic processes are completely at the mercy of the external temperature. There is ample evidence for adaptation of these organisms to warmer or cooler temperatures in such a way as to result in much the same level of activity regardless of the external temperature. As Barcroft (*38*) stated, "It is clear that nature has learned so to exploit the biochemical situation so as to escape from the tyranny of a simple application of the Arrhenius equation. She can manipulate living processes in such a way as to rule, and not be ruled by, the obvious chemical situation."

Although many examples of biochemical and physiological change in response to temperature are known, the molecular basis of these adaptations is still little understood. The generalization may be made that an organism adapted to low temperatures has a higher metabolism at a particular temperature than one acclimated to high temperatures. This holds for adaptations of members of the same population in the laboratory and for adaptations to different climatic conditions among different populations of the same species in nature. A large number of examples of such adaptations has recently been discussed by Bullock (*37*).

Adaptive regulation may occur at the molecular, cellular, organ, or behavioral level. While the underlying biochemical mechanisms are not well known, it is evident that these must include (*a*) a mechanism for perception of absolute temperature, as distinguished from temperature differences, and (*b*) a compensation system. The temperature-sensing mechanism may lie in the rate of discharge of nerve fibers, although this is not definitely established (*12, 39*). The compensatory mechanisms appear to be varied. In reptiles, which show little capacity for physiological adaptation (*37*), compensation is accomplished on the behavioral level, by moving into and out of the sunshine, for example. Hormonal control is undoubtedly a factor. Thyroxine has been shown to affect the temperature adaptation of frog's heart (*38, 40, 41*). The metabolism of isolated organs is frequently found to be adapted in the same way as that of the intact animal from which they were derived (*42, 43, 44*). However, in some cases certain organs may show temperature adaptation and others not (*43, 44*).

The activity of various enzymes in cell or tissue homogenates has been found to vary with the temperature to which the organism was adapted. To cite a few examples, there was an increase in the dehydrogenase activity of breis from goldfish liver tissue, snail salivary and

midgut glands, kidney and muscle tissue from earthworm and potato beetle, cell material from *Escherichia coli* and the yeast *Torulopsis*, when the individuals or cell populations had been kept at low temperatures (*12, 45–48*). On adaptation to higher temperatures, alkaline glycerophosphatase activity decreases in the potato beetle, while in yeast lactoflavin content decreases, pyridine nucleotide increases, peroxidase and catalase increase (*45, 49, 50*). Amino acid decarboxylase activity in *E. coli* and *Micrococcus lysodeikticus* decreases with increasing adaptation temperature (*51*). The temperature maxima for enzyme activity in homogenates or breis generally move up and down with the previous temperature to which the organism was acclimated, just as do the maxima of the over-all respiratory process. Temperature maxima for cell proliferation show no such differences, however (*12, 37, 45, 52*).

It should be emphasized that these phenomena are changes in activity within the normal temperature range for growth of the organism, not alterations which permit the organism to adjust to extremes. The latter will be discussed in Sections III and IV.

B. Homoiotherms

The transition from poikilothermy to homoiothermy, i.e., the utilization of metabolic energy to provide an internal environment of constant temperature, is probably one of the most important evolutionary discoveries made by the higher animals. It must have had a great adaptative advantage to succeed in spite of being at first glance a very dangerous transition, involving the abandonment of the mechanism which regulated enzymatic activity in response to temperature variation and staking the life of the organism on its ability to maintain its temperature within narrow limits. To quote Barcroft again (*38*), "Here then is a very fine issue—the cold-blooded animal successfully adopting ingenious mechanisms, first biochemical, then physiological, in order to adapt its heart to the variations of its environment; the warm-blooded animal discarding what its cold-blooded predecessor has laboriously beaten out, invoking the nervous system to reverse the normal biochemical relationship and gaining a new freedom by adapting, not itself to the internal environment, but the internal environment to itself."

Three factors are involved in this internal temperature regulation: (1) an increase in the rate of output of metabolic energy to cope with the demands made upon it for heating; (2) the elaboration of structures which act as effective heat exchangers; (3) development of a control center which regulates the production of energy for heating and the heat exchange devices for heating and cooling.

The differences in energy output between some representative homo-

TABLE II

ENERGY PRODUCTION BY SOME REPRESENTATIVE HOMOIOTHERMS AND POIKILOTHERMS[a]

Animal	Body weight (kg.)	Body surface area (square meters)	Energy output[b]	
			(cal./kg./day)	(cal./square meter/day)
HOMOIOTHERMS				
Man	56–65	1.65–1.83	23.2–25.5	790–910
Baboon	6.2	0.40	48	760
Chimpanzee	38	1.1	29.2	980
Macaque	4.2	0.31	49.3	675
Rhesus monkey	3.2	0.26	48.4	610
Beef cattle	400–500	3.2–4.7	15.2	1635
Sheep	42.7–49.5	0.95–1.1	25.7–26.3	1160–1180
Swine	250	3.2	15.8	1230
Shetland pony	280	4.4	16.7	1060
Dog	11.7–15.5	0.58–0.65	33.5–38.5	770–800
Cat	3.0	0.2	50	750
Rabbit	3.5	0.2	47	810
Rat	0.2	0.03	130	830
Mouse	0.02	0.005	170	525
Guinea pig	0.8	0.07	62	690
Marmot	2.6	0.18	28.3	420
Elephant	3670	23.8	13.3	2060
Chicken	2.3	0.19	78	940
Ring dove	0.15	0.03	130	700
Duck	0.93	0.1	90	855
Goose	3.3–5.0	0.23–0.29	54–61	880–940
Parakeet	0.03	0.009	225	690
Pigeon	0.28	0.04	100	670
Canary	0.016	0.006	310	760
POIKILOTHERMS				
Lizard	1.2	0.11	2.5	29
Turtle	0.14	0.02	11.4	64
Frog	0.05	—	—	130
Toad	0.05	—	—	130
Sturgeon	1400	11.8	0.3	31

[a] Data from W. S. Spector, ed., "Handbook of Biological Data." National Academy of Sciences–National Research Council, Washington, D. C., 1956.

[b] The energy output values represent basal metabolism.

iotherms and poikilotherms are shown in Table II. The much higher values for the homoiotherms are clear. Moreover, the energy output of poikilotherms usually varies directly with temperature, becoming higher as the temperature is increased. An outstanding characteristic of homo-

iotherms, on the other hand, is their inverse temperature coefficient of energy output. Within the temperature range in which their temperature regulating mechanism can function heat production becomes less as the external temperature rises. The difference between the two types of organisms is shown graphically in Fig. 2.

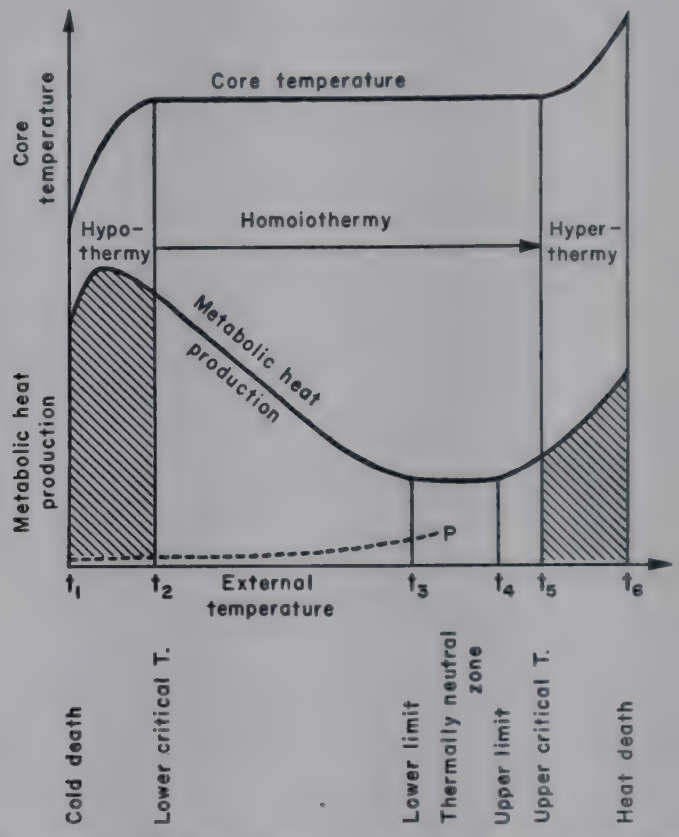


FIG. 2. General pattern of heat production and variation in body temperature of homoiotherms exposed to various external temperatures. By "core temperature" is meant the temperature of the main part of the body, which is not affected by temperature variations that may occur in the extremities. The dotted curve *P* shows, for comparison, the heat production of a poikilothermic animal. [After Hensel (*12*)].

This reversed temperature relationship calls for a greater energy output of the homoiotherms, and consequently a greater demand for food compared to the poikilothermic forms. In small birds and mammals, e.g., hummingbirds and shrews, almost continual feeding is required to compensate for the high heat loss due to their high surface/volume ratio (*53*). When not feeding, the body temperature of these animals drops and they sink into a torpid state. Larger forms, however, may maintain their body temperature for days during starvation, even though this requires burning much of their body substance as fuel (*54*).

The rate of metabolism is inversely proportional to the body size. For example, the metabolism of the shrew, *Sorex cinereus*, at 24–28° is 65 times that of a man and 200 times that of an elephant (*12*). There is some question as to what measure of body size should be taken when a quantitative comparison of the relation between size and metabolism is made. The rule $Q = kG^n$, where Q is the total heat formation per unit

time, k a constant depending on the shape of the animal, G the body weight, and n an exponent which varies from 0.75–0.82, has been found to hold by several investigators (*55, 56, 57*). If the differences in metabolic intensity were purely to compensate for differences in heat loss due to differences in surface/volume ratio, as was originally postulated by Rubner (*58*), then the value of n would be 0.67. The higher values of n indicate a more complex relationship than that predicted by the surface rule.

Discussion of the various structures developed for insulation and heat exchange by homoiotherms, or of the nervous and hormonal factors involved in temperature control, would involve a major digression into mammalian physiology and lead far from the field of biochemical responses to thermal energy (cf. Hensel, *12*, for example, for discussions of these subjects). Here, it will suffice to mention that temperature control appears to be one of the more advanced and delicate functions of homoiotherms, and one which can at times be lost temporarily, e.g., in hibernating animals, or in cats under deep narcosis (*37*).

III. Organisms with Unusual Thermal Requirements

A. Cryophils

The ability to live at extremely low temperatures, often below the freezing point of water, is found among a number of plants, microorganisms, and poikilothermic animals (homoiothermic organisms living at subzero temperatures pose a problem in insulation and heat exchange rather than biochemistry). Siberian larches survive temperatures as low as −67.8° (*12*). Several herbaceous plants can carry out photosynthesis at −2 to −3°, and respiration at −6 to −7° (*59*). Algae in polar seas live at even lower temperatures (*60*). Some insects can survive at −20 to −30° (*61*), and are found active at as low as −6° in snow and glacial ice (*62*). Various bacteria are notable for their ability to metabolize and develop at subzero temperatures in media such as foodstuffs, where freezing is prevented. To cite some examples, members of the *Pseudomonas* group were isolated from glacier water by Schmelz in 1888 (*63*). In the same year Fischer (*64*) isolated cryophilic representatives of various bacteria from sea water, soil, and foods. Bacteria will grow in ice cream at as low as −10° (*65*). Horowitz-Wlassowa and Grinberg (*66*) observed among microbes capable of growth at −4 to −7° various cocci, spore-forming rods, a few yeasts, and many molds. The multiplication of these microorganisms was very slow at the subzero temperatures; many of them had optimal temperatures around 37°. Conn (*67*) found that freezing temperatures in soil still permitted extensive bacterial development.

The mold *Sportrichium carnis* will grow at −10° (68); as will *Choetostylum* and *Hormodendrum* (69); *Chadosporium* will grow and complete its life cycle at −7.8°. Development of bacteriophage in cryophilic bacteria was observed by Elder and Tanner (70).

Growth at subzero temperatures often leads to morphological changes in microorganisms. On return to normal temperatures the usual morphology is re-established (66, 71).

No examples are known of organisms which develop better at subzero temperatures than in the normal temperature range. As noted above, forms which can grow at exceptionally low temperatures usually do better at slightly higher ones. Their adaptation merely allows them to endure better the damaging effects of cold. These may include the formation of ice in and around cells, structural alterations in the protoplasm, and accumulation of toxic products due to decreased solubility of many compounds in the surrounding medium or to differential slowing of metabolic reactions, with consequent build-up of intermediates. The activation energy of some enzyme reactions changes markedly around 0° (2). In plants, low temperatures may cause a water and salt imbalance because of their effect on transpiration (12, 72).

Increased cold resistance has been associated by Precht (12) with an increase in the osmotic concentration of the cell contents, a decrease of free water and an increase in bound water within the cell, and a decrease in the permeability of the cell membrane. Not all of these effects are necessarily associated with a particular case of cold resistance. The primary consideration is that freezing of the cell contents be prevented. It may be noted that the same adaptations confer on the organism an increased resistance to drying. For relevant literature on the relation between cold injury and dehydration cf. Luyet and Gehenio (73).

The surrounding medium greatly affects the ability of microorganisms to develop at low temperatures. Inorganic ions have been reported to increase cold resistance of yeast in the order Li < Ca < Mg < Na < K (74). High concentrations of sugars and other dissolved materials favor growth at low temperatures (75, 76, 77). Colloids in the medium play an important role in increasing cold resistance (74, 75, 78, 79, 80). Foodstuffs are thus favorable media for low-temperature growth of microorganisms.

B. Thermophils

Considerably more biochemical interest has been attracted by those organisms which have the ability, and in some cases the necessity, to live at temperatures which result in the death of other forms of life. Al-

Of these are microorganisms, including blue-green algae, bacteria, actinomycetes, and fungi. The massive development of thermophilic blue-green algae which occurs in many hot springs early attracted the attention of naturalists, but these photosynthetic thermophils have been the subjects of few biochemical studies.* The investigation of the physiological and biochemical factors which permit growth at high temperatures has for the most part been carried out with the spore-forming bacteria, which are readily accessible to laboratory study, and which grow at temperatures at least as high as 55°.

The first thermophilic bacterium was isolated by Miquel (*82, 83*) from water of the Seine in 1879. In spite of the cool habitat from which it was obtained, it was able to grow at 73°, and could not grow at low temperatures. During the following decades, thermophilic bacteria were found in almost every sample of soil, mud, or water which was examined. They were isolated not only from tropical soils (*84, 85*) and desert sands (*86*), but also from air (*83, 87*), freshly fallen snow (*88, 89*), sea water (*90*), and even ocean bottom mud (*91*). Water (*92–95*), sewage (*92, 96*), feces of man and various animals (*83, 90, 97–103*), cultivated soils (*90, 100, 102, 104–107*), and masses of decaying plant materials (*108–115*) were especially good sources. The great majority of the bacteria isolated at high temperatures proved to be aerobic sporeforming bacteria, nonsporeformers are rare and facultative forms more common than obligate anaerobes, although the latter are known (*116–118*).

Mass development of thermophilic bacteria often occurs in tightly packed masses of plant materials, such as hay or manure piles. In haystacks, for example, high temperatures are likely to develop, even to the point where the hay spontaneously ignites. This has been shown to be the result of bacterial action; the rise in temperature is accompanied by a loss in weight of the hay, and gaseous products and volatile acids are formed (*112, 113, 119, 120*). Alteration of plant material by thermophilic bacteria is sometimes used in the preparation of certain types of fodder or manure (*121–123*).

In general, however, thermophilic bacteria are a nuisance rather than a help in agricultural and industrial practice. Thermophilic sporeformers are among the principal causes of spoilage of canned foods, since not only are their vegetative cells adapted to life at elevated temperatures, but also their spores are unusually heat resistant, some

* The "thermophilic" strains of *Chlorella pyrenoidosa* which have recently been reported (*81*) have a temperature maximum only slightly above 40°. The only chlorophyte known which can grow at a temperature of 55° is *Cyanidium caldarium* (*81a*).

surviving half an hour of autoclaving (*124–129*). The bacterial count of milk sometimes increases during pasteurization, due to development of thermophils during the heating process (*128, 130–133*). Canned milk is also subject to coagulation by thermophilic bacteria (*134, 135*). Thermophilic sporeformers are often a problem in sugar refineries, where they have been observed living and fermenting in syrups containing up to 40% sucrose (*125, 126, 135–139*). Other thermophils cause a ropy spoilage of bread (*140*).

The principal biochemical activities which have been shown to be carried out by thermophilic bacteria are the digestion of starch and proteins (*87, 90, 92, 101, 128, 141–148*), and the fermentation of sugars to acidic products (*128, 138, 140–143*). Crude cultures of thermophils have been shown to decompose cellulose, to denitrify, and to fix nitrogen (*144, 145, 149–155*). Thermophilic bacteria which can reduce sulfate to sulfide as their major hydrogen acceptor are known (*156*), as are high temperature thiobacilli (*157*). A wide variety of carbon sources can be utilized by thermophilic sporeformers (*5*). In general, it may be stated that most, if not all, of the major types of metabolism carried on by bacteria which live at ordinary temperatures may also be found among the thermophils.

Since all the available biochemical evidence indicates a close relationship between thermophilic microorganisms and those living at more ordinary temperatures, there is little reason to consider them as survivors of a previous geological era (*158, 159*), or visitors from another planet (*160*). It is, however, necessary to explain their occurrence in habitats such as mud and sea water, which are known to have been at low temperatures for long periods of time. The most likely explanation of the ubiquitous occurrence of thermophilic microorganisms is their selection, as variant forms of the natural mesophilic population, or as mutants induced by the high temperature, whenever the requisite thermal conditions are supplied.

Although Tsiklinsky (*103, 107*) had obtained a thermophilic variant of a mesophilic *Bacillus subtilis* strain, and Dallinger (*161, 162*) had patiently adapted a protozoan to growth at 70°, the first extensive support for the idea that naturally occurring thermophilic microorganisms are derived from mesophilic ones came from the work of Lieske (*163*) with actinomycetes. His experimental evidence was not especially strong, but his conclusions have been strengthened by the work of Kluyver and Baars (*156*) (cf. also Clegg, *164*) with *Desulfovibrio desulfuricans* and that of Allen (*5*) with aerobic sporeforming bacteria. These authors were able to convert mesophilic bacteria into thermophilic ones in the laboratory. It seems probable that the transition from mesophily to

thermophily usually involves selection of mutants rather than gradual adaptation of a population. Kluyver and Baars used large inocula in their experiments, and those of Allen were designed for mutant selection. A number of attempts which have been made to increase the temperature range for growth of bacterial cultures by gradual adaptation have been unsuccessful (*88, 165*). Sobotka *et al.* (*166*) have recently used the selection principle to obtain soil bacteria with exceptionally high temperature maxima.

IV. Methods of Protection against the Destructive Effects of Thermal Energy

The destructive effects of thermal energy are most apparent in the upper portion of the biokinetic temperature range, and the means of protection against such effects are often most clearly illustrated by the thermophilic microorganisms. The external environment of the cells, structural features of the organisms, including structural modifications on a molecular level, and dynamic effects of metabolism in preventing or repairing thermal damage all play a part in permitting these organisms to contend with high temperatures.

As with cold resistance, the thermal resistance of microorganisms depends upon the environment in which they are placed; the most obvious example of this being the well known fact that bacteria in a dry condition can survive heat which is lethal when they are wet. Examples indicating an effect of the chemical composition of growth media upon the heat resistance of organisms are readily found. Nakamura (*167*) in 1897 reported that beer yeast, which completely lost its ability to ferment on heating for 30 minutes at 50° in water or 10% sucrose solution, retained its fermenting ability if heated in a sugar solution containing the minerals necessary for growth, or in meat extract. The ability to reproduce was lost in all cases. Göbel (*168*) obtained greater survival of *Pseudomonas aeruginosa* in bouillon than in physiological salt solution when the two suspensions were exposed to the same elevated temperature. Richet *et al.* (*169*) increased the maximum growth temperature of a lactic acid bacterium from 36–37° to 41–42° by cultivation in the presence of 3.4% KCl.

Although Casman and Rettger (*165*) failed to find any protective effect of hypertonic solutions on a number of organisms of the *B. subtilis* group, Baumgartner (*170, 171*) reported that 0.5 *M* solutions of glucose, galactose, lactose, maltose, sucrose, glycerol, and mannitol protected *E. coli* from thermal destruction. Anderson *et al.* (*172*) found that glucose and sucrose increased the heat resistance of *B. coagulans*, while NaCl and several organic acids decreased it.

Van Halteren (*173*) has shown that yeast is protected against the effects of heat by concentrated solutions of various substances. Yeast heated for one hour at 44° in the presence of 1 or 2 *M* NaCl, 1, 2, or 3 *M* glucose, or 30% sucrose showed little, if any, decrease in respiration and was able to assimilate nitrogen immediately on being returned to 37°. On the other hand, yeast heated in a dilute medium containing glucose, minerals, and succinate buffer had a reduced oxygen uptake and nitrogen assimilation did not proceed for several hours after the period of heating.

The medium may increase heat resistance by providing conditions for active metabolism, so that the organisms are better able to repair the damage done by heat, it may aid growth at high temperatures because it contains substances which protect proteins from thermal denaturation, or it may affect the water relations of the cell. The first effect may readily be observed with thermophilic bacteria of the *B. subtilis* group. Unless these bacteria are placed in an environment which permits active metabolism, their thermal resistance is not noticeably greater than that of analogous mesophilic organisms (*4*, *5*).

As to the second possibility, i.e., the effects of sugars, neutral salts, and indifferent proteins in preventing enzyme inactivation, Virtanen (*174*) found that the proteinase of *Ps. fluorescens* is not inactivated by 10-minutes boiling in a medium containing additional soluble protein, but is readily destroyed at 60° in a protein-free medium. Bacterial lipases behaved similarly. Maas (*175*) found that a mutant of *E. coli* which requires pantothenic acid for growth above 30° contained an extraordinarily thermolabile pantothenate-synthesizing enzyme. This enzyme was rapidly inactivated even at 25°, but could be protected by sugars or neutral salts.

There are some indications of an effect of divalent ions on thermal resistance. The decrease in growth factor requirements of some thermophilic sporeformers in media containing relatively high concentrations of Ca or Mg ions is suggestive of a protective effect. Bacterial spores, the most thermoresistant living objects known, have a high Ca content (*176*). Calcium has been observed to increase the thermal resistance of *Paramecium* (*177*). The bacteriophages of *E. coli*, which are notably thermoresistant in a suitable medium (*178*) are very sensitive to the effects of heat unless divalent ions are present in the medium (*179*).

Although exceptions are known, most organisms which live at high temperatures are small in size, at least in the sense of having slender cells, and a high surface/volume ratio. Several years ago Lamanna (*180*) pointed out that size decreases as temperature range for growth rises in the genus *Bacillus*. A survey of the members of the

blue-green algal genera *Phormidium* and *Oscillatoria* described by Copeland (*181*) in his monograph on the Yellowstone thermal Myxophyceae indicates that the same rule holds with these organisms; those inhabiting hot waters are, almost without exception, small-celled types (cf. Table III). Thermophilic actinomycetes are, like their mesophilic relatives, of small diameter (*163*). Among the fungi, however, cells 10 μ and more in diameter can exist at high temperatures (*182*). If the correlation be-

TABLE III

COMPARISON OF DIAMETERS OF MESOPHILIC AND THERMOPHILIC MEMBERS OF THE GENERA *Phormidium* AND *Oscillatoria* FOUND IN YELLOWSTONE PARK

Organism	Diameter (μ)
Forms growing at 70° and above	
Phormidium geysericola	0.4–0.6
Phormidium bijahensis	0.3–0.5
Oscillatoria filiformis	0.4–0.5
Forms not found above 40°	
Phormidium yellowstonense	6.8
Phormidium fragile	1.2–2.3
Phormidium foveolarum	approx. 1.5
Phormidium luridum	1.7–2.0
Oscillatoria limosa	11–23
Oscillatoria anguina	6–8
Oscillatoria princeps	29–42
Oscillatoria sancta	10–20
Oscillatoria lacustris	5–7
Oscillatoria amphibia	2–3
Oscillatoria amoena	2.4–5
Oscillatoria splendida	2–3
Oscillatoria boryana	6–8

tween high-temperature growth and high surface/volume ratio is anything more than a coincidence, then, since a large surface/volume ratio should be favorable for rapid movement of substrate into the cell and of waste products out of it, rapid exchange of materials with the environment must be considered of importance for organisms developing at elevated temperatures.

Much more attention has been given to what may be considered as structural adaptations on the molecular level, such as changes in protein configuration resulting in an increased stability at high temperatures. Quite early in the investigation of thermophilic microorganisms it was found that hydrolytic enzymes resistant to high temperatures could be separated from the culture filtrates of thermophilic bacteria. Oprescu (*147*) obtained an amylase preparation which was still active after heating for a half hour at 85°, although the proteinase activity of his

filtrates was lost during heating for one hour at 60°. Pringsheim (*154, 155*) studied a cellulase from thermophilic cellulose-fermenting bacteria which hydrolyzed cellulose to cellobiose over the temperature range 20–70°. These findings lent support to the view that the proteins of thermophilic microorganisms must be in some way modified so that they are not destroyed by the high temperatures at which the organisms grow. Recently Sobotka *et al.* (*166*) have studied the extracellular proteins produced by some thermophilic bacteria and found them to be resistant to thermal denaturation. It is of special interest that the protein from organisms grown at 75° contained an amino acid component not present in protein formed by cells growing at lower temperatures.

As knowledge of enzymes and microbial biochemistry increased, it became evident that extracellular hydrolytic enzymes need not be good indicators of the thermostability of the essential intracellular proteins of microorganisms. Attention then turned to the respiratory enzymes and other cellular proteins. Militzer and his co-workers (*183–187*) have furnished good evidence for the existence of thermostable respiratory enzymes in a stenothermal thermophilic bacterium. This group has obtained thermostable cell-free preparations of malic dehydrogenase, cytochrome oxidase, cytochrome b, cytochrome c, succinoxidase, aldolase, and apyrase. Most of these enzymes are found together in a particulate "red fraction" which appears to constitute a morphological entity within the cell, but a soluble apyrase has been prepared, indicating that combination in a particle is not necessary for thermostability of the proteins from their bacterium.

Koffler and his collaborators (*188–190*) have shown that the total cytoplasmic protein and the flagella from some thermophilic bacteria are more heat stable than similar preparations from bacteria growing at lower temperatures. Flagella from thermophils also showed an increased resistance to urea, acetamide, and detergents, indicating more "effective" hydrogen bonding in proteins from the organisms living at high temperatures. This work is of interest in that it provides the first indication of how proteins from thermophilic bacteria might be rendered more thermostable, and perhaps also gives a clue as to the reason for the inability of stenothermal thermophils (the bacteria in which notably heat resistant proteins have been demonstrated are generally stenothermal, see below) to grow at ordinary temperatures. The greater rigidity given to their proteins by a greater degree of hydrogen bonding may render them unable to carry out their normal catalytic functions until a greater than usual thermal energy is provided. However, individual enzymes of stenothermal thermophils can function at ordinary temperatures.

Reactions of enzyme inactivation and protein denaturation are char-

acterized by a large contribution of the entropy term to the free energy of activation (*6*, *7*). This offsets the high values for the heat of activation, and makes it possible for these reactions to occur at relatively low temperatures. The entropy of activation, in turn, depends upon the change from order to disorder which occurs when the first few bonds are broken (*6*, *7*). These bonds are considered to be hydrogen bonds (*7*), hence a change in the degree of "effectiveness" of hydrogen bonding might be expected to have marked effects on the ease of thermal denaturation of proteins.

Thermolabile enzymes have been found, however, even in stenothermal thermophilic microorganisms. Gaughran (*191–193*) studied the effect of temperature on the activity of the complete respiratory system and of catalase, cytochrome oxidase, and several dehydrogenases from five stenothermal thermophils. The curves for the activity of all the enzymes studied indicated appreciable inactivation at temperatures from 37 to 50°, although the bacteria grew well at these temperatures. Militzer *et al.* (*187*) found that the pyruvic oxidase from the same thermophilic bacterium which had yielded a number of thermostable enzymes was thermolabile outside the cell, although fairly stable in the intact organism.

Certain workers (*193–198*) have emphasized the role played by the degree of saturation of the protoplasmic lipids in determining the temperature range for growth of organisms. Measurements have shown that an organism, whether a bacterium or a homoiothermic animal, grown at a high temperature has more saturated fatty acids in its lipids than one grown at a low temperature. This result has been interpreted in terms of the need for maintaining a certain protoplasmic viscosity if vital processes are to continue. Its meaning for the functioning of the organism remains, however, somewhat obscure.

That thermostable proteins play an important role in permitting some microorganisms to live at unusually high temperatures is evident from the preceding discussion. The question may be asked, however, whether other factors do not make equally important contributions to the adjustment of the organisms to these extreme conditions. There is a considerable body of evidence which indicates that this question should be answered in the affirmative.

The eurythermal thermophilic bacteria do not possess markedly thermostable proteins (*4*, *5*), yet are able to grow at temperatures up to 60–65°. Both stenothermal and eurythermal thermophils die rapidly in the absence of nutrients at temperatures which permit good growth when proper nutritional conditions are maintained (*4*, *5*). Thermophilic bacteria do not show the stationary phase characteristic of bacteria

growing at lower temperatures (*4, 5, 199*). Cessation of growth is accompanied by rapid death and lysis of the cells (cf. Fig. 3). Even during active growth there is evidence for the death of a part of each generation of cells before division can occur (*5*).

These results have been taken as evidence that active metabolism is important in maintaining life at high temperatures; that, in spite of the possession of thermostable proteins by some bacteria, damage is done to the cell by growth at elevated temperatures, and that this damage must be continually repaired if the cell is to continue to live (*4, 5, 165*).

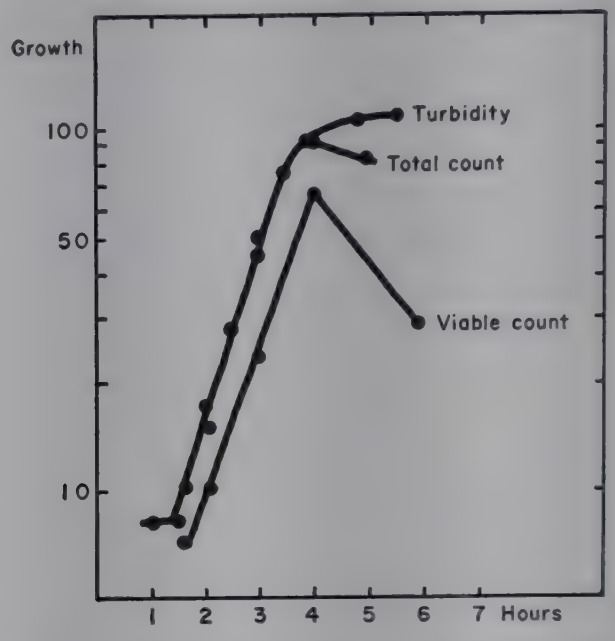


FIG. 3. Growth of a thermophilic bacterium as measured by turbidity, total cell count, and viable cell count (*5*). The viable count was lower than the total count throughout the growth period, and fell rapidly when active growth ceased. Turbidity is measured on an arbitrary scale. A reading of 100 corresponds to 3×10^8 cells per milliliter.

If this hypothesis is correct, the nutritional requirements of microorganisms might be expected to become more exacting as the temperature of growth is raised. There are a number of cases in which this has been observed. *E. coli* grown at 44° required glutamic acid or nicotinic acid, neither of which was required for growth at 37° (*200*). *Pasteurella pestis* required a supplement of alanine, leucine, serine, threonine, biotin, and pantothenate for growth at 36° instead of 28° (*201*). *Coprinus fimetarius* required methionine at 35–40° but not at 25° (*202*). The effect of growth temperature on the nutritional requirements of a number of sporeforming bacteria is given in Table IV. It will be seen that some, but not all, become nutritionally more exacting at higher temperatures. High concentrations of nutrients have been shown to favor high temperature growth of both bacilli and *Euglena* (*203*).

TABLE IV

INFLUENCE OF TEMPERATURE ON THE NUTRITIONAL REQUIREMENTS OF THERMOPHILIC BACTERIA, ACCORDING TO CAMPBELL AND WILLIAMS[a]

Organism	Nutritional requirements at		
	36°	45°	55°
Bacillus coagulans 2	Histidine Thiamine Biotin Folic acid	Same as at 36°	Same as at 36°
Bacillus coagulans 1039	Thiamine Biotin Folic acid	Same as at 36°	Histidine Methionine Thiamine Niacin Biotin Folic acid
Bacillus coagulans 32	Methionine Tryptophan Thiamine Biotin	Histidine Methionine Tryptophan Thiamine Biotin Folic acid	Same as at 45°
Bacillus stearothermophilus 1356	No growth	Leucine Thiamine Niacin Biotin	Same as at 45°
Bacillus stearothermophilus 3084	Biotin Folic acid	Same as at 36°	Thiamine Biotin Folic acid
Bacillus stearothermophilus 4259	Biotin Folic acid	Methionine Histidine Niacin Biotin Folic acid	Same as at 45°
Bacillus stearothermophilus 5149–5	Methionine Thiamine Biotin Folic acid	Biotin Folic acid	Same as at 45°

[a] L. L. Campbell and O. B. Williams, *J. Bacteriol.* **65**, 141–145 (1953).

It may also be mentioned that the curves for killing of bacteria are logarithmic with time (*204*, *205*). This sort of curve has been interpreted as evidence that death occurs as the result of the destruction of a few key catalysts in each cell, rather than denaturation of large

numbers of protein molecules (5, 205, 206). The suggestion has been made that the key catalysts are those which govern the synthesis of protein (5). Some recent results by Hancock (206), who measured protein synthesis in a thermophilic sporeformer, are in agreement with this hypothesis.

V. Thermal Energy as a Factor in Evolution

Thermal energy has probably been a factor in the evolutionary picture from the time of the development of the first living things. Many enzyme-catalyzed reactions will also proceed at a measurable rate in the absence of catalysts, or with smaller, less efficient organic molecules as catalysts. It seems probable that primitive forms of life, appearing in a world which may have been higher in temperature than it is at present and not being subjected to the biological competition which today places a high premium on biochemical efficiency, may well have carried out many reactions without enzyme catalysis, drawing from the environment sufficient thermal energy to provide the activation energy for these reactions. [There is, however, no reason to consider present-day thermophilic organisms as survivors of such primitive forms of life. The enzymatic apparatus of present-day thermophils appears as complex as that of organisms living at lower temperatures (5, *12*)].

After the fundamental patterns of biochemical organization of present-day living organisms had developed, thermal energy, or the lack of it, continued to play an evolutionary part by demanding the development of numerous biochemical and physiological mechanisms in order to enable living creatures to survive in environments of diverse, and in some cases apparently unfavorable, temperature conditions (cf. Section II, A). This development continued to such an extent that organisms are found at subzero temperatures and at temperatures only a little below the boiling point of water (Section III). The "higher" animals, however, have evolved different methods for coping with temperature changes and extremes. They have developed mechanisms for maintaining the body temperature constant, thus eliminating the need for biochemical adjustment to changing temperature (Section II,B).

Addendum

Two contributions summarizing recent work on the biochemistry of adaptation of organisms to extreme temperatures have appeared since the survey of literature for this paper was completed at the end of 1957.

The metabolic changes that occur as warm-blooded animals adapt to a cold environment were discussed in a recent symposium (207). The

rincipal overall change in metabolism that is observed is an increase i heat production. Hormones of the pituitary, adrenal, and thyroid lands are involved in this process in a complex manner. Since it is nown that these hormones can cause changes in the level of various nzymes in the animal, Potter (*207*) has suggested that metabolic cold daptation is due to a change in hydrogen transport from the usual athway to one or more alternative pathways, such as TPN oxidation, n which phosphorylation does not occur efficiently and more energy is issipated as heat.

At the other end of the biokinetic temperature range, Kurtz (*205*) as considered the problem of heat tolerance in plants. Desert plants an survive and grow at temperatures similar to those endured by hermophilic microorganisms. For example, *Opuntia* does not cease rowth until its internal temperature reaches 62°. After exposure to uch temperatures, growth resumes on cooling to 50°. Seeds of saguaro actus remain viable after 7 days at 83°. The author cites a number of xamples in which the ability to synthesize an essential metabolite is ne factor controlling the temperature range for growth in plants and uggests that differences in heat tolerance between plants might derive rom the temperature characteristics of their synthetic enzymes, as has reviously been suggested for thermophilic microorganisms (*4*, *5*).

References

1. A. K. Balls and H. Lineweaver, *Food Research* **3**, 57–67 (1938).
2. I. W. Sizer and E. S. Josephson, *Food Research* **7**, 201–209 (1942).
3. V. P. Maier and A. L. Tappel, *Anal. Chem.* **26**, 564–566 (1954).
4. M. B. Allen, *J. Gen. Physiol.* **33**, 205–214 (1950).
5. M. B. Allen, *Bacteriol. Revs.* **17**, 125–173 (1953).
5a. H. Eyring, R. P. Boyce, and J. D. Spikes, this volume, Chapter 2
6. I. W. Sizer, *Advances in Enzymol.* **3**, 35–62 (1943).
7. A. E. Stearn, *Advances in Enzymol.* **9**, 25–74 (1949).
8. H. Eyring, *J. Chem. Phys.* **3**, 107–115 (1935).
9. H. Eyring and A. E. Stearn, *Chem. Revs.* **24**, 253–270 (1939).
10. F. W. Went, in "Vernalization and Photoperiodism, a Symposium" (F. Verdoorn, ed.), pp. 145–157. Chronica Botanica, Waltham, Mass., 1948.
11. F. W. Went, "The Experimental Control of Plant Growth" Chronica Botanica, Waltham, Mass., 1957.
12. H. Precht, J. Christophersen, and H. Hensel, "Temperatur und Leben." Springer, Berlin, 1955.
13. I. D. Strelnikov, *Compt. rend.* **192**, 1317–1319 (1931).
14. P. Krueger and F. Dumpiva, *Biol. Generalis* **9**, 165–168 (1933).
15. H. Krogerus, *Acta Zool. Fennica* **53**, 1–157 (1948).
16. D. A. Parry, *J. Exptl. Biol.* **28**, 445–462 (1951).
17. E. B. Edney, *J. Exptl. Biol.* **30**, 331–349 (1953).
18. B. P. Uvarov, *Trans. Roy. Entomol. Soc. (London)* **79**, 1–247 (1931).
19. C. Vorbrodt, *Intern. Entomol. Z.* **15**, 162–169, 173–179, 202–206; **16**, 10–14.

30–31, 46–47, 62–63, 81–83, 97–99, 113–115, 129–131, 145–147, 161–162 (1922).
20. E. Handschin, *Rev. suisse zool.* **27,** 65–98 (1919).
21. P. A. Buxton, *Proc. Roy. Soc.* **B96,** 123–131 (1924).
22. H. Giersberg, *Z. vergleich. Physiol.* **7,** 657–695 (1928).
23. G. Huber, *Ber. deut. botan. Ges.* **50,** 68–76 (1932).
24. G. Fritsche, *Beih. Botan. Zentr.* **50,** 251–322 (1933).
25. P. Portier, "Physiologie des Animaux Marins." Flammarion, Paris, 1938.
26. D. L. Gunn, *Biol. Revs.* **17,** 293–314 (1942).
27. F. G. Benedict, *Carnegie Inst. Wash. Publ.* No. 425 (1932).
28. A. Hase, *Naturwissenschaften* **14,** 995–997 (1926).
29. J. E. Morrow, Jr., and A. Mauro, *Copeia* pp. 108–116 (1950).
30. B. Huber, "Der Wärmehaushalt der Pflanzen," pp. 10–15. Datterer, Freising, Germany, 1935.
31. K. Hummel, *Metereol. Rundschau* **1,** 147–150 (1947).
32. A. Herfs, Höfchenbriefe f. Wissenschaft und Praxis, No. 3, 1950.
33. E. B. Edney, *J. Exptl. Biol.* **28,** 271–280 (1951).
34. K. Koidsumi, *Mem. Fac. Sci. Agr. Taihoku Imp. Univ.* **12,** 1–16 (1935).
35. M. E. Davies and E. B. Edney, *J. Exptl. Biol.* **29,** 571–582 (1952).
36. H. Ullrich, *Klin. Wochschr.* pp. 189–192 (1944).
37. T. H. Bullock, *Biol. Revs.* **30,** 311–342 (1955).
38. J. Barcroft, "Features in the Architecture of Physiological Function." Cambridge University Press, London and New York, 1934.
39. Y. Zotterman, *Ann. Rev. Physiol.* **15,** 357–372 (1953).
40. T. J. B. Stier and H. E. Taylor, *J. Cellular Comp. Physiol.* **14,** 309–312 (1938).
41. C. L. Smith, *J. Exptl. Biol.* **28,** 141–164 (1951).
42. C. N. Peiss and J. Field, *Biol. Bull.* **99,** 213–224 (1950).
43. J. A. Freeman, *Biol. Bull.* **99,** 416–424 (1950).
44. J. L. Roberts, *Proc. 19th Intern. Physiol. Congr. Montreal* pp. 706–707 (1953).
45. H. Precht, *Verhandl. deut. zool. Ges. Marburg* **1950,** 179–180 (1950).
46. H. Precht, *Biol. Zentr.* **70,** 71–85 (1951).
47. J. Christophersen and H. Precht, *Biol. Zentr.* **71,** 313–326 (1952).
48. C. Kirbergen, *Z. vergleich. Physiol.* **35,** 175–198 (1953).
49. J. Christophersen and H. Precht, *Biol. Zentr.* **69,** 240–256 (1950).
50. K. Marzusch, *Z. vergleich. Physiol.* **34,** 75–92 (1952).
51. E. F. Gale and H. M. R. Epps, *Biochem. J.* **36,** 600–618 (1942).
52. J. Christophersen and H. Precht, *Biol. Zentr.* **69,** 300–323 (1950).
53. F. Groebbels, *Pflügers Arch. ges. Physiol.* **218,** 98–114 (1927).
54. E. Streicher, D. B. Hackel, and W. Fleischmann, *Am. J. Physiol.* **161,** 300–306 (1950).
55. M. Kleiber, *Physiol. Revs.* **27,** 511–541 (1947).
56. P. R. Morrison, *J. Cellular Comp. Physiol.* **31,** 281–291 (1948).
57. O. P. Pearson, *Science* **108,** 44 (1948).
58. M. Rubner, *Z. Biol.* **19,** 535–562 (1883).
59. O. Zeller, *Planta* **39,** 500–526 (1951).
60. H. Kylin, *Arkiv Botanik* **10,** 1–37 (1910).
61. R. W. Salt, *Can. J. Comp. Med.* **13,** 177–181 (1949).

62. W. Speyer, *Entomologie, Wiss. Forschungsber. Naturwiss. Reihe* **43**, 43–48 (1937).
63. L. Schmelz, *Zentr. Bakteriol.* **4**, 545–547 (1888).
64. Fischer, *Zentr. Bakteriol.* **4**, 89–92 (1888).
65. J. Weinzirl and A. E. Gerdeman, *J. Dairy Sci.* **12**, 182–190 (1929).
66. L. M. Horowitz-Wlassowa and L. D. Grindberg, *Zentr. Bakteriol. Parasitenk. II* **89**, 54–62 (1933).
67. H. J. Conn, *Zentr. Bakteriol. Parasitenk. II* **28**, 422–434 (1910).
68. R. B. Haines, *J. Exptl. Biol.* **8**, 379–388 (1931).
69. C. Bidault, *Compt. rend. soc. biol.* **85**, 1017–1018 (1921).
70. A. L. Elder and F. W. Tanner, *Proc. Soc. Exptl. Biol. Med.* **24**, 645 (1926–1927).
71. E. Almquist, *Zentr. Bakteriol. Parasitenk. I Orig.* **48**, 175–186 (1909).
72. H. Ullrich, *Protoplasma* **38**, 165–183 (1944).
73. B. J. Luyet and P. M. Gehenio, "Life and Death at Low Temperatures." Biodynamica, Normandy, Mo. 1940.
74. B. Stille, *Arch. Mikrobiol.* **14**, 554–587 (1950).
75. S. C. Keith, *Science* **37**, 877–879 (1913).
76. C. M. Hilliard and M. A. Davis, *J. Bacteriol.* **3**, 423–431 (1918).
77. V. H. McFarlane, *Food Research* **5**, 43–57, 59–68 (1940); **6**, 481–492 (1941); **7**, 508–518 (1942); **8**, 67–77 (1943).
78. A. Goetz and S. S. Goetz, *Proc. Am. Phil. Soc.* **79**, 361–370 (1938).
79. W. Stiles, *Protoplasma* **9**, 459–468 (1930).
80. W. Kessler and W. Ruhland, *Planta* **28**, 159–204 (1938).
81. C. Sorokin and J. Myers, *Science* **117**, 330–331 (1953).
81a. M. B. Allen, *Rapp. et communs. 8^me congr. intern. botan.* Sect. 7, pp. 41–42 (1954).
82. P. Miquel, "Les organismes vivantes de l'atmosphère," thesis, University of Paris, 1883.
83. P. Miquel, *Ann. Micrographie* **1**, 3–10 (1888).
84. E. de Kruijff, *Bull. Dept. Agr. Indes Neerl.* No. 30, 1–21 (1909).
85. E. de Kruyff, *Zentr. Bakteriol. Parasitenk. II* **26**, 65–74 (1910).
86. L. Negre, Contribution a l'étude des microbes thermophiles. Etude biologique de la flore bacterienne thermophile du Sahara," thesis, University of Paris, 1918.
87. T. Sames, *Z. Hyg.* **33**, 313–362 (1900).
88. S. M. Golikowa, *Zentr. Bakteriol. Parasitenk. II* **69**, 178–184 (1926).
89. L. Rabinowitsch, *Z. Hyg.* **20**, 154–164 (1895).
90. A. MacFadyen and F. R. Blaxall, *J. Pathol. Bacteriol.* **3**, 87–99 (1894); *Trans. Jenner Inst. Preventive Med.* **2**, 162–187 (1899).
91. J. W. Bartholomew and S. C. Rittenberg, *J. Bacteriol.* **57**, 658 (1949).
92. P. Bardou, "Étude biochimique de quelques bactériaceés thermophiles et de leur rôle dans la désintégration des matières organiques des eaux d'égout," thesis, Lille, 1906; *Zentr. Bakteriol. Parasitenk. I Ref.* **39**, 744–745 (1907).
93. D. Benignetti, *Riv. igiene sanit. publ.* **16**, 449–455 (1905).
94. P. Georgevitch, *Zentr. Bakteriol. Parasitenk. II* **27**, 150–167 (1910).
95. L. E. Morrison and F. W. Tanner, *J. Bacteriol.* **7**, 343–366 (1922).
96. E. Eckelmann, *Zentr. Bakteriol. Parasitenk. II* **48**, 140–178 (1917).
97. A. Ambroz, *Zentr. Bakteriol. Parasitenk. I Ref.* **48**, 257–270, 289–312 (1911).

98. N. N. Antischkow, *Zentr. Bakteriol. Parasitenk. I Orig.* **41,** 326–332, 426–43 (1906).
99. D. H. Bergey, *J. Bacteriol.* **4,** 301–306 (1919).
100. L. A. Black and F. W. Tanner, *Zentr. Bakteriol. Parasitenk. II* **75,** 360–37 (1928).
101. G. Bruni, *Zentr. Bakteriol. Parasitenk. I Orig.* **38,** 177–185, 298–307 (1905
102. Globig, *Z. Hyg.* **3,** 294–321 (1888).
103. P. Tsiklinsky, *Ann. inst. Pasteur* **17,** 217–240 (1903).
104. A. Certes and Garrigou, *Compt. rend.* **103,** 703–706 (1886).
105. W. A. Feiter, *Soil Sci.* **23,** 47–56 (1927).
106. P. van Tieghem, *Bull. soc. botan. France* **28,** 35–36 (1881).
107. P. Tsiklinsky, *Ann. inst. Pasteur* **13,** 788–795 (1899).
108. F. Cohn, *Ber. deut. botan. Ges.* **11,** (66)–(69) (1893).
109. C. Dupont, *Compt. rend.* **134,** 1449–1451 (1902); *Ann. agron.* **28,** 289–31 (1902).
110. A. B. Griffiths, *Chem. News* **70,** 273–275 (1894).
111. H. Hoffmann, *Z. Spiritusind.* **20,** 287, 320, 337, 344, 369, 384, 402, 41 (1897).
112. R. Medem, *Jahrb. deut. landw. Ges.* **9,** 40–60 (1894).
113. H. Miehe, "Die Selbsterhitzung des Heues." Fischer, Jena, 1907.
114. S. A. Waksman, T. C. Cordon, and N. Hulpoi, *Soil Sci.* **47,** 217–224 (1939
115. S. A. Waksman and T. C. Cordon, *Soil Sci.* **47,** 217–224 (1939).
116. S. R. Damon, and W. A. Feiter, *J. Bacteriol.* **10,** 37–46 (1925).
117. L. S. McClung, *J. Bacteriol.* **29,** 173–187 (1935).
118. F. S. Paine, *Zentr. Bakteriol. Parasitenk. II* **85,** 122–129 (1931).
119. F. W. J. Boekhout and J. J. Ott de Vries, *Zentr. Bakteriol. Parasitenk. II* 13 675–681 (1904); **18,** 27–29 (1907); **21,** 598–607 (1908); **23,** 106–10 (1909).
120. M. Duggeli, *Naturwiss. Z. Forst- u. Landwirtsch.* No. 11; *Zentr. Bakteri Parasitenk. II* **18,** 688–689 (1906).
121. A. Dunez, *Ann. agron.* **3,** 505–521 (1933).
122. G. Ruschmann, *Zentr. Bakteriol. Parasitenk. II* **77,** 216–239 (1929).
123. B. B. Stoller, F. B. Smith and P. E. Brown, *J. Am. Soc. Agron.* **29,** 717–72 (1937).
124. E. J. Cameron and J. R. Esty, *J. Infectious Diseases* **39,** 80–105 (1926).
125. E. J. Cameron and C. C. Williams, *Zentr. Bakteriol. Parasitenk. II* **76,** 28–3 (1928.)
126. F. M. Clark and F. W. Tanner, *Food Research* **2,** 27–39 (1937).
127. L. Moller, *Compt. rend. soc. biol.* **99,** 641–642 (1928).
128. F. Schandinger, *Z. Untersuch. Nahr- u. Genussm.* **19,** 865–880 (1903).
129. W. Verhoeven, *Antonie van Leeuwenhoek J. Microbiol. Serol.* **16,** 269–28 (1950).
130. M. O. Eckford, *Am. J. Hyg.* **7,** 201–221 (1927).
131. F. Janke, *Milchwirtsch. Forsch.* **6,** 303–350 (1928).
132. C. Lehmann, *Landwirtsch. Ver.-Sta.* **43,** 373–388 (1894).
133. A. H. Robertson, *N. Y. State Agr. Expt. Sta. Tech. Bull.* No. 130 (1927).
134. R. V. Hussong and B. W. Hammer, *J. Bacteriol.* **15,** 179–188 (1928).
135. F. B. Culton, *Ind. Eng. Chem.* **28,** 1235–1238 (1936).
136. E. J. Cameron, *Food Inds.* **9,** 182–183 (1937).
137. E. J. Cameron and W. D. Bigelow, *Ind. Eng. Chem.* **23,** 1330–1333 (1931
138. O. Laxa, *Zentr. Bakteriol. Parasitenk. II* **4,** 362–367 (1898).

139. O. Lara, *Zentr. Bakteriol. Parasitenk. II* **6**, 288–295 (1900).
140. V. Harasto, *Ann. Acad. Sci. Fennicae* **A53**, 1–114 (1939).
141. C. Coolhaas, "Bijdrage tot de kennis der dentrificatie van verzuurde gronden en Koolhydraten door thermophiele bacterien," Dissertation, Wageningen, Holland 1927.
142. C. Coolhaas, *Zentr. Bakteriol. Parasitenk. II* **75**, 161–170, 344–360 (1928).
143. V. Kerko, *Ann. Acad. Sci. Fennicae* **A21**, 1–125 (1923).
144. P. de Kruijff, *Bull. Dept. Agr. Indes Neerl.* No. 30, 1–21 (1908).
145. P. de Kruijff, *Zentr. Bakteriol. Parasitenk. II* **26**, 65–74 (1910).
146. G. Michaelis, *Arch. Hyg.* **26**, 285–293 (1896).
147. V. Opresco, *Arch. Hyg.* **33**, 164–186 (1898).
148. A. Schillinger, *Hyg. Rundschau* **8**, 569–570 (1898).
149. K. F. Kellerman and I. C. McBeth, *Zentr. Bakteriol. Parasitenk. II* **34**, 485–494 (1912).
150. A. Kruisz, *Zentr. Bakteriol. Parasitenk. II* **36**, 338–349 (1912).
151. E. Muchtenin, *Zentr. Bakteriol. Parasitenk. II* **66**, 325–344 (1926).
152. V. Omeliansky, *Zentr. Bakteriol. Parasitenk. II* **8**, 256–264, 353–361 (1902).
153. H. Pringsheim, *Zentr. Bakteriol. Parasitenk. II* **31**, 23–27 (1911).
154. H. Pringsheim, *Z. physiol. Chem.* **78**, 266–281 (1912).
155. H. Pringsheim, *Zentr. Bakteriol. Parasitenk. II* **38**, 513–516 (1913).
156. A. J. Kluyver and J. K. Baars, *Proc. Koninkl. Ned. Akad. Wetenschap* **35**, 370–378 (1932).
157. M. B. Allen, unpublished data.
158. W. H. Wood, *Am. Naturalist* **23**, 394–400 (1889).
159. A. Ambrosi, *Zentr. Bakteriol. Parasitenk. II* **37**, 3–18 (1913).
160. S. Arrhenius, *Z. physik. Chem.* **130**, 316–319 (1927).
161. W. H. Dallinger, *J. Roy. Microscop. Soc.* **3**, 1–16 (1880).
162. W. H. Dallinger, *J. Roy. Microscop. Soc.* **7**, 185–199 (1887).
163. R. Lieske, "Morphologie und Biologie der Strahlenpilze," Borntraeger, Leipzig, 1921.
164. L. F. L. Clegg and S. E. Jacobs, *Symposia Soc. Gen. Microbiol.* **3**, 308–325 (1953).
165. E. P. Canman and L. F. Berger, *J. Bacteriol.* **26**, 77–123 (1933).
166. H. Sobotka, H. Baker, A. V. Lunada-Opper, and S. H. Hutner, *Rev. fermentations et ind. aliment.* **12**, 51–56 (1957).
167. T. Nakamura, *Bull. Imp. Univ. Tokyo Coll. Agr.* **3**, 237 (1897); *Zentr. Bakteriol. Parasitenk. II* **4**, 777–778 (1897–1898).
168. R. Göbel, *Z. Hyg.* **100**, 380–387 (1923).
169. C. Picket, E. Bachrach, and H. Cardot, *Compt. rend.* **180**, 1937–1938 (1925).
170. J. G. Baumgartner and M. D. Wallace, *J. Soc. Chem. Ind.* **53**, 294T–297T (1934).
171. J. G. Baumgartner, *J. Bacteriol.* **36**, 369–382 (1938).
172. E. E. Anderson, W. B. Bowden, Jr., and C. B. Fellers, *Food Research* **14**, 499–510 (1949).
173. P. van Halteren, *Bull. soc. chim. biol.* **32**, 458–463 (1950).
174. A. I. Virtanen, *J. Bacteriol.* **28**, 447–460 (1934).
175. W. K. Maas, *Federation Proc.* **11**, 475 (1952).
176. H. R. Curran, B. C. Brunstetter, and A. T. Myers, *J. Bacteriol.* **45**, 485–494 (1943).
177. H. M. Chalkley, *Physiol. Zool.* **3**, 425–440 (1930).

178. S. A. Koser, *J. Bacteriol.* **13,** 14–15 (1927).
179. O. Maaloe, personal communication.
180. C. Lamanna, *J. Bacteriol.* **39,** 593–596 (1940).
181. J. J. Copeland, *Ann. N. Y. Acad. Sci.* **36,** 1–232 (1936).
182. K. Noack, *Jahrb. wiss. Botan.* **51,** 593–648 (1912).
183. C. E. Georgi, W. Militzer, L. Burns, and J. Heotis, *Proc. Soc. Exptl. Biol. Med.* **76,** 598–601 (1951).
184. C. Marsh and W. Militzer, *Arch. Biochem. Biophys.* **36,** 269–275 (1952).
185. W. Militzer, T. B. Sonderegger, L. C. Tuttle, and C. E. Georgi, *Arch. Biochem.* **24,** 75–82 (1949).
186. W. Militzer, L. C. Tuttle, and C. E. Georgi, *Arch. Biochem. Biophys.* **31,** 416–423 (1951).
187. W. Militzer and L. Burns, *Federation Proc.* **11,** 260 (1952).
188. H. Koffler and H. Gale, *Arch. Biochem. Biophys.* **67,** 249–251 (1957).
189. J. Adye, H. Koffler, and G. E. Mallett, *Arch. Biochem. Biophys.* **67,** 251–253 (1957).
190. G. E. Mallett and H. Koffler, *Arch. Biochem. Biophys.* **67,** 254–256 (1957).
191. E. R. L. Gaughran, "Growth, Respiration, and Chemical Composition of the Stenothermophilic Bacteria with Particular Reference to Temperature," Ph.D. thesis, Massachusetts Institute of Technology, Cambridge, Massachusetts, 1946.
192. E. R. L. Gaughran, *J. Gen. Physiol.* **32,** 313–327 (1949).
193. E. R. L. Gaughran, *J. Bacteriol.* **53,** 506 (1947).
194. J. Belehradek, "Temperature and Living Matter." Borntraeger, Berlin, 1935.
195. G. Fraenkel and H. S. Hopf, *Biochem. J.* **34,** 1085–1092 (1940).
196. L. K. Pearson and H. S. Raper, *Biochem. J.* **21,** 875–879 (1927).
197. E.-F. Terroine, R. Bonnet, G. Kopp, and J. Vechot, *Bull. soc. chim. biol.* **9,** 605–620 (1927).
198. E.-F. Terroine, C. Hatterer, and P. Roehrig, *Bull. soc. chim. biol.* **12,** 682–702 (1930).
199. A. Imsenecki and L. Solnzeva, *J. Bacteriol.* **49,** 539–546 (1945).
200. G. C. Ware, *J. Gen. Microbiol.* **5,** 880–884 (1951).
201. E. D. Spure and G. M. Hills, *J. Gen. Microbiol.* **5,** viii (1951).
202. L. Fries, *Physiol. Plantarum* **6,** 551–563 (1953).
203. H. Baker, S. H. Hutner, and H. Sobotka, *Ann. N. Y. Acad. Sci.* **62,** 349–376 (1955).
204. O. Rahn, *J. Gen. Physiol.* **13,** 179–205 (1929).
205. O. Rahn and W. R. Schroeder, *Biodynamica* **3,** 199–208 (1941).
206. R. Hancock, *J. Gen. Microbiol.* **17,** 480–490 (1957).
207. L. D. Carlson, J. S. Hart, P. F. Scholander, C. P. Lyman, V. R. Potter, and D. J. Ingle, *Federation Proc.* **17,** 1044–1073 (1958).
208. E. B. Kurtz, Jr., *Science* **128,** 1115–1117 (1958).

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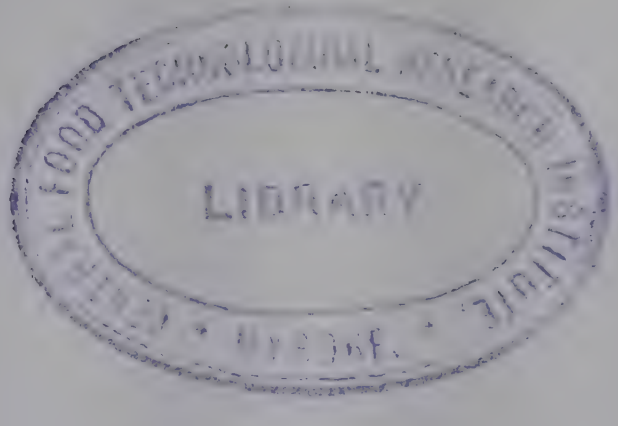
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